INDUSTRIAL RESEARCH ASSOCIATIONS: SOME TAXATION PROBLEMS

IN discussing recently the report of the Pritchard Committee on the 'rates' charged by local government authorities against charities and kindred bodies in Britain, passing reference was made to the position of the industrial research associations (*Nature*, **184**, 1343; 1959). It was stated that the Committee had heard oral evidence from the Parliamentary and Scientific Committee urging that a new statutory relief of 75 per cent should be allowed to industrial research associations. The report notes that under section 449 of the Income Tax Act, 1952, industrial research associations enjoy the same reliefs from income tax as do charities, but the Pritchard Committee submits that the research associations are much more akin to the research establishments of individual firms in industry than to charities. Accordingly, the Committee recommended that the relief considered appropriate for research establishments conducted by individual firms within the curtilage of their industrial hereditaments should be extended to premises of industrial research associations.

The Pritchard Committee, which rightly points out that the treatment for rating of separate hereditaments occupied by individual firms for industrial research is outside its terms of reference, claimed that its view in this matter corresponds to that expressed in evidence by the Department of Scientific and Industrial Research. The report, however, does not indicate the nature of the argument submitted for a new statutory relief of 75 per cent, and in particular, Lieut.-Commander Christopher Powell, secretary of the Parliamentary and Scientific Committee, has pointed out to the Editors that research associations do not at present enjoy the benefit of industrial de-rating. In the view of the Parliamentary and Scientific Committee they should enjoy that benefit, and the figure of 75 per cent recommended was chosen because, when the evidence was submitted, industrial de-rating amounted to 75 per cent.

The memorandum submitted in evidence by the Parliamentary and Scientific Committee points out that research associations do not at present enjoy this benefit of industrial de-rating, because their premises do not fall within the definition of the class of industrial hereditaments comprising 'factories' and 'workshops' in that they are not conducted "by way of trade or for purpose of gain". There is the additional doubt whether 'manual labour' is exercised as required by the definition, when regard is had to the nature of the duties of laboratory workers and research assistants. The research associations might have taken up the matter when legislation affording rating relief to industry was under review, but at the time and until 1948 they were under the impression that they would continue to enjoy the benefits of the Scientific Societies Act.

While it is true that the research associations, which at present enjoy no rating relief of any kind, will obtain 50 per cent relief from rates if the proposals of the Pritchard Report are implemented, so long as that is the percentage of relief accorded to industry, the undoubted anomaly will remain that premises of a commercial concern on which research and development work is carried on may obtain partial exemption from rates, while those of a scientific research association as such obtain none. Associated with this anomaly is the fact that if industrial research and development work by a commercial concern is carried out in a factory hereditament where the predominant activity is pr duction, it can come under the umbrella of industrial de-rating relief afforded to the factory as a whole, but if it is carried out in a separate hereditament full rates have to be borne on that hereditament. The Parliamentary and Scientific Committee submitted that the correct action is to put all premises of the commercial concern in which industrial research and development work proceeds on the same footing regardless of whether or not they form part of the factory.

This is so obviously the logical policy that there can be little doubt that it expresses the general view of the scientist and technologist, and it should be pressed again when the proposals of the Pritchard Committee come before Parliament. Indeed, it is reasonable to claim that research associations have an even stronger claim to rating relief than premises on which a commercial concern conducts research and development work. As the Parliamentary and Scientific Committee urged in its evidence, the research associations do not make profits and cannot pass on the increased cost of their work to the public as can a commercial concern which sells its products. They have educational functions in connexion with training schemes, and their activities educate their member firms in science and technology, sometimes also incidentally, it might be noted, assisting the supply of technically trained staff. Much of the outcome of their work is published in the scientific Press, and their services to technological education should not be overlooked. Notwithstanding that their primary duty is to serve the industries which contribute to their support, their fundamental aim is to see that newly won scientific and technological knowledge is applied throughout British industry.

These public benefits, the extent of which depends on the magnitude of the financial support the associations receive, are every whit as worthy of consideration as their contribution to keeping their industries in the forefront of scientific achievement, which is the fundamental reason why relief from rates is claimed for them. The Parliamentary and Scientific Committee recognizes that privately owned commercial concerns benefit substantially from the work of the research associations, and for that reason does not claim full exemption in the way that it is claimed for scientific societies and institutions. It

makes instead the sound claim that the public benefit arising from the work of the research associations justifies relief of 75 per cent from rates, and this relief should stand, independent of whether or not industrial de-rating generally is continued. To that extent, and apart from the specific figure recommended, the Parliamentary and Scientific Committee goes appreciably further than the Pritchard Committee, the report of which does not indicate the full considerations on which the claim is based.

WHITE WILDERNESS

Arctic Wild

By Lois Crisler. Pp. xii+274+60 photographs. (London: Martin Secker and Warburg, Ltd., 1959.) 25*s*. net.

LOIS CRISLER, courageous wife of a professional camera-man engaged in filming arctic wild life for a well-known film company, lived with her husband for two summers and one winter, in tent or flimsy hut, almost entirely alone in the barren wilderness of the Brooks Range in north-western Alaska. During most of this time the two saw no human beings other than the bush pilot who supplied them with the essentials of life at long and irregular intervals, when the weather or the landing conditions did not make his hazardous flights too risky. But they shared a mutual sympathy with, and a dedicated understanding of, the wild life around them. To these two, the tundra, mountains and rivers, in all their changing aspects through the seasons, meant an opportunity to know life much nearer to the primitive than most educated, civilized human beings could ever hope to experience.

To enable them to work unhindered by interference from the occasional Eskimo hunter, and to study and film the undisturbed caribou and wolves, the area in which they lived and worked was made a temporary sanctuary. They themselves carried no firearms. One result of their sojourn in the wilderness, and perhaps the most important one, is this book. It is the work of a writer dedicated to the cause of the conservation of the wild places of the Earth and of the wild animals without the presence of which the face of the Earth remains empty and dead. The picture presented here, in terse but vivid language, is of the wild life of a region that modern man, with his craze for killing and destruction, has still scarcely scratched—the last wilderness of North America. But the frightening hint we are given of what the future holds for this land is depressing enough. As the Crislers flew south from Alaska, their work finished, the mass invasion of the Far North by the military and other personnel engaged in the creation of the *DEW*-Line for the radar defence of the continent was bringing into the wilderness thousands of outsiders to whom any living thing was a target and nothing more, to be killed or wounded indifferently.

To the naturalist, and particularly to the animal behaviourist, this book is a revelation. The main objective of the Crislers was to observe and film, in all possible aspects, the interwoven lives of the caribou in their great migrating herds, and of their attendant predators, the wolves. These are the two most important large mammals of the north. The observations of the writer and her husband form the first clear-cut account of the relationship of the two species. They found that the fit and healthy caribou, even the quite young fawns, were able to outdistance the hunting wolves with comparative ease. In almost all cases investigated it was found that the caribou killed were those hampered by disease, old age, or injury of some kind. The natural role of the wolf, in fact, is as the culler of the unfit, Nature's method of limiting the undue multiplication of the herds. How delicate the balance, and how disastrous the effects of interference with it, is shown by the quoted instance of the effect of wolf 'control' on the Nelchina herd of Alaskan caribou a few years earlier. From an estimated total of 4,000 animals, in ten years the figure, without the limiting factor of wolf predation, had risen to the startling total of about 10,000. The available winter range was no longer sufficient, and the consequent trampling and over-grazing threatened the livelihood of the excessive caribou population. Yielding to wiser counsels, the U.S. Fish and Wildlife Service reversed its former policy and gave complete protection to the wolves in that area. Elsewhere in Alaska a 50-dollar bounty on each wolf sets every man's hand against them and poisons men's minds against their continued existence.

The Crislers' attitude to wolves provides the meat for the most engrossing chapters of this book. They tell of the hand-rearing of wild wolf cubs, and the subsequent sharing of their lives. First two cubs were reared to maturity, and in the second season an entire litter of five, dug out of their den, were brought up by hand and allowed to develop their individual personalities as naturally as possible. In both cases the young wolves regarded the Crislers as their parents, learnt to accompany them on their daily outings on the tundra, to return home after a day's freedom to the pen which was their life's centre, to romp and play, roughly but without malice, with their human substitutes for wolf parents. Naturally this gave unequalled opportunities for detailed study of wolf psychology and behaviour patterns. The results, told with a complete and refreshing absence of the usual animal behaviourist's technical jargon, provide an enthralling story. The excellent photographs, showing adult wolves playing boisterously with their 'foster parents', indulging in communal howling practice with the humans joining in, or accompanying them on their caribou camera hunts, show that the text does not exaggerate the astonishing degree of trust and friendship between wolves and humans. There is rich material here for the student of animal behaviour, and particularly for the student of the origins of the domestication of the dog, to which the wolf is demonstrably so closely akin. The description of the male and female adult wolves, themselves not yet mated, adopting the litter of five young cubs and making it their business to provide all their food in the form of caribou meat carried home in the wolves' stomachs to be disgorged in front of the cubs, is a striking example of the inherent selflessness and gentleness of wolves towards young not even their own.

This is probably the most important study of wolf personality and reactions that has yet been made. I could only wonder at and admire the unafraid trust placed by the Crislers, alone, unarmed, and 90 miles from the nearest help, in these adult wolves, living a practically free and uncontrolled life, yet looking to these two humans for leadership, companionship and play. They admit, casually enough, that at times they had doubts about the reliability of their wolves, but the only moments of trouble and stress came when unfamiliar sights, sounds and persons troubled

the animals. To them any break with the familiar pattern of their lives spelt insecurity and danger, and they reacted accordingly.

It will be a very long time before a book as sympathetic to wild life as this (yet without sentimentality or anthropomorphism), and at the same time so readable, is likely to appear. The only criticism I have to make is that the provision of a map would have helped readers to follow the Crislers' journeys and references to Alaskan place-names connected with their work.

R. W. HAYMAN

PREHISTORY OF SOUTHERN RHODESIA

Inyanga

Prehistoric Settlements in Southern Rhodesia. By Roger Summers. With contributions by H. B. S. Cooke, P. V. Tobias, H. Wild, J. F. Schofield and K. R. Robinson. Pp. xviii+336+22 plates. (Cambridge: At the University Press, 1958. Published for the Inyanga Research Fund.) 50*s*. net.

ROGER SUMMERS and his collaborators here describe the fruit of six months field-work under the auspices of the Inyanga Research Fund during 1949–51. Their object was to discover the age and purpose of the stone-walled ruins and terraces that cover between two and three thousand square miles on the Inyanga Highlands of Southern Rhodesia. Summers and his co-workers found that the walled settlements, now in ruin, were built by a prehistoric Iron Age community that displaced the Stone Age inhabitants of Inyanga apparently between the seventh and fourteenth centuries A.D. The reader may ask why we use terms like 'Stone Age' and 'prehistoric' for such recent times, but it must be remembered that, until the nineteenth century, most of Africa south of the Sahara either remained untouched by the great civilizing events that brought writing to the Mediterranean world, or reflected them in part only.

Our previous knowledge of the ruins depended on a few short papers. Now Roger Summers has given us a full monograph, while specialists contribute the latter half of the book on other aspects of the archæology, botany, surface geology, animal and human palæontology of the area. Their enthusiasm carried them beyond the limits of the Iron Age, down into Stone Age depths, for K. R. Robinson contributes a chapter on people who lived in Inyanga long before the Iron Age stone-wall builders arrived there.

Summers's account of the Iron Age at Inyanga is based on no less than thirty-six excavations within an area of 800 square miles. I congratulate him on achieving the aims of the sponsoring Fund, and presenting such an interesting account of the framework of the thriving population that based its way of life on the terraces, trackways, water furrows, stone buildings and pottery it left in this region. Here, too, is an account of the metal-producing techniques that supplied the spears, axes, hoes and knives necessary to the economy and its defence; and also the east coast trade that for centuries poured millions of glass beads and other objects into African Iron Age communities. These beads give some indication of absolute dating, as J. F. Schofield's valuable chapter suggests; but in general these things have an age-range too wide for any but the broadest dating.

P. V. Tobias interprets the two Iron Age skeletal remains as representing a Negro and a Boskopoid–Negro hybrid, anatomical characters found in the present-day Bantu-speaking population in Rhodesia. There may be little doubt that the Iron Age remains in Inyanga are due to their ancestors, and not to any exotic people.

Summers distinguishes two main Iron Age cultures in Inyanga. The earlier cultures may have flourished between the seventh and eighth centuries A.D., but could be as late as the fourteenth century A.D. The scarcity of foreign imports supports the earlier date; indeed, accurate radiocarbon age estimation may show it to be even earlier. Recent radiocarbon dates for Iron Age settlements in Northern Rhodesia suggest that metal-producing farmers may have arrived in the area centuries earlier than our previous conjectural dating based mainly on imported beads.

The later cultures produced the astonishing terraces, stone-lined pits, roadways and water-channels which are such striking features of the Inyanga landscape, as aerial photographs in the book show. These Iron Age people had a thorough grasp of soil conservation, for they made miles of contoured terraces for grain fields and built massive stone walls across gullies to stop soil erosion.

Summers gives a study of the environmental background to Iron Age life, but the reconstruction of climatic change on the basis of erosion and deposition is considered uncertain in view of recent South African research which, in my opinion, has done much to disprove the traditional hypotheses of this kind. Few specialists would accept a chronology based on the hypothetical climatic changes Summers proposes in his concluding chapters.

The archæological remains described in this book are as impressive as Zimbabwe, but it is unfortunate that Summers omits clear comparison with that well-known centre and its associated settlements. I hope he will give us a third book relating Inyanga and Zimbabwe to each other and to all other Iron Age remains in Rhodesia that interest both the archæologist and the general observer of African achievement in the past.

The book reaches the usual high standards of the Cambridge University Press; the line drawings are excellent, but the photographs could have been improved by 'bleeding' the blocks for reproduction.

REVIL MASON

EVOLUTION FOR THE GENERAL READER

Nature and Man's Fate

By Prof. Garrett Hardin. Pp. xii+375. (New York: Rinehart and Company, Inc., 1959.) 6 dollars.

IN their introductory remarks the publishers of Prof. Hardin's book on evolutionary theory and the course of human evolution describe it as "challenging", "provocative" and "controversial". Even though words of this kind have been used so much that they have lost a great deal of their original force, there is little doubt that "Nature and Man's Fate" will bring pleasure to all those who prefer to see the truth set down in terms of black and white.

The plan of the book is on orthodox lines. The emergence of the theory of evolution as set out by Charles Darwin and A. R. Wallace is preceded by accounts of earlier theories and the intellectual and

emotional atmosphere which existed when "The Origin of Species" was published. This is followed by the story of how evolutionary theory gathered momentum and support with the re-discovery of the Mendelian laws of heredity. The latter part of the book explores the significance of genetics in human life and development. In general, the historical and factual part of Hardin's book is remarkably good, mainly because of the interesting and often novel anecdotes and reminiscences which the author produces to support the theories and philosophies of the leading *personalia*.

When Hardin begins to speculate, however, he adopts the overbearing attitude so typical of those who see the world devoid of any shade of grey. Darwin is chided for his lack of mathematics, his support of pangenesis, and his inability "to discover genetics". All Lamarkists are banished from court. "Samuel Butler and St. George Mivart were intensely proud and personally ambitious far beyond their small abilities". It would be interesting to hear Hardin's assessment of a well-known English professor of zoology who advocates that more may be heard of the inheritance of acquired characteristics when the possibilities of cytoplasmic inheritance have been further explored. In his attack on the Soviet attitude to genetics—which is weakened when Hardin adopts the approach of a journalist rather than a man of science—Needham, Bernal and Haldane are accused of something almost verging on intellectual dishonesty. All this to support Hardin's strongly held view that what evolutionary theory and genetic laws have taught us is the essential need for competition if human life is to go on.

It is a pity that the author has spoilt what might have been a good book with over-protestation. "Nature and Man's Fate" should bring him much publicity but little credit. T. H. Hawkins

HEAT TRANSFER AND HEAT EXCHANGERS

Heat Transfer

Vol. 2. By the late Max Jakob. With the technical and editorial assistance of Prof. S. P. Kezios. Pp. xxxii+652. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1957.) 120*s*. net.

An Introduction to Fluid Mechanics and Heat Transfer

With Applications in Chemical and Mechanical Process Engineering. By Prof. J. M. Kay. Pp. xvi + 309. (Cambridge: At the University Press, 1957.) 37*s*. 6*d*.

Heat Exchangers

Applications to Gas Turbines. By Dr. W. Hryniszak. Pp. xii+343. (London: Butterworths Scientific Publications; New York: Academic Press, Inc., 1958.) 63*s*.; 10 dollars.

THE second volume of the late Prof. M. Jakob's treatise on "Heat Transfer", completed under the guidance of his colleague, Prof. S. P. Kezios, maintains the very high standard set by the first. The first volume left over for consideration the solution of the equations for radiant heat transfer in non-absorbing and absorbing media, a topic which occupies the first quarter of this second volume. The greater part of the book deals with selected fields of application in heat-transfer engineering, discussing first the well-established topics by giving a mathematical treatment of recuperative and regenerative heat exchangers and cooling tower theory. There follows consideration of the newer branches of study in the science of heat transfer, including transpiration cooling and cooling by falling liquid films, heat transfer problems encountered at high fluid velocity through laminar and turbulent boundary layers, and concluding with chapters on heat transfer in liquid metals and in packed columns. A supplement brings up to date some of the material in Volume 1, though none of the many literature references given throughout the book is carried beyond 1953, and indeed those relating to regenerator theory do not go beyond 1940. Presentation of further material is held over to a later edition of the treatise. The two volumes together form an indispensable work of reference for the libraries of all heat transfer laboratories.

Prof. Kay's book is based directly on a university course of lectures in chemical engineering. Picking up this book after Prof. Jackob's two volumes, one wonders how the author has compressed into a modest volume an introduction both to heat transfer and fluid mechanics. This has been achieved by an admirably concise mathematical treatment of his material, but also by omitting entirely consideration of radiant heat transfer and any solution of the unsteady state conduction equations. The particular strength of the book lies in the successful manner in which it inter-relates and interweaves the phenomena of fluid flow, mass transfer and heat transfer. The author makes use of vector notation to an extent which need not cause undue difficulty to one to whom it is unfamiliar, though it seems inappropriate to introduce the simple concept of thermal resistance via equations written in vector notation. While there are a few short chapters at the end of the book dealing very briefly with applications in process engineering, the absence of any worthwhile mention of two-phase flow problems is surprising in a book written for chemical engineers. One suspects that this is because their study lends itself less readily to the neat and tidy mathematical treatment which features so largely in this book.

The book by Dr. Hryniszak is in a different category from the two others. While Prof. Jakob's is likely to appeal most to the postgraduate student and research worker and Prof. Kay's to the more able undergraduate, Dr. Hryniszak's would make little appeal to either, for it is essentially a book for the specialist in gas-turbine heat exchangers. Although the non-specialist student might find some interest in the sections dealing with the mechanical aspects of recuperative and regenerative heat exchangers, he would find little attraction in the manner of presentation of the theoretical matter; for in an attempt to keep the mathematics as simple as possible, the theoretical relations are frequently presented as complicated formulæ with little in the way of derivation. Consequently, the contents of the book too often resemble extracts from a design manual, and such manuals seldom make exciting reading. Only the specialist will have the patience and inclination to dig deeper if he wants to compare the author's methods with his own. The volume deals not only with the heat exchanger but also, in optimization procedures, treats it as an integral part of the gas-turbine plant. R. W. Haywood

Pictorial History of Philosophy

By Dagobert D. Runes. Pp. x+406. (New York: Philosophical Library, 1959.) 15 dollars.

THIS handsome volume includes approximately 1,000 portraits, photographs, facsimiles, archæological illustrations and other pictorial material connected in one way or another with philosophy. The net is cast very widely; Goethe, Hitler, Buddha, massacres of the Jews by the Crusaders, photographs of practices by adherents of Yoga, compete for attention with the more conventional practitioners of the craft, such as Aristotle, Cicero, Duns Scotus, Husserl, Wittgenstein and Dewey. Although the principles of exclusion are not obvious, the effect is to have here a collection of philosophical portraits of a range which it would be hard to find elsewhere. Title-pages from important classics are liberally spread throughout the volume, as well as dramatic scenes from the general history of thought and religion, for example, Calvin arguing with Farel, alchemical laboratories, the dying Seneca, Pericles addressing the Athenians. There is less to be said for the literary entries that accompany the illustrations. They vary from a sixth to a third of one page, Kant getting rather less than, say, Carlisle, Croce or Russell, and too frequently mention what is trivial and biographical at the expense of an accurate appraisal of a person's thought. The page facing Russell's portrait shows Stalin, and that with Heidegger has Hitler's portrait: the author makes no bones where his sympathies lie; deeply pro-Jewish, with a full page for "Lincoln, Sage and Seer", and a penchant for the wisdom of the East. Perhaps, though this may introduce an amateurish note, the feelings that emerge are none the worse for it; and if one would not want to learn one's philosophy from it, one must be grateful for the very many rare photographs such as a daguerreotype of Schopenhauer or others of Lloyd Morgan, Alexander, Bradley, Bosanquet; all reproduced with magnificent quality.

GERD BUCHDAHL

Theory and Applications of Nuclear Induction

By Prof. Ajit Kumar Saha and Tara Prasad Das. Pp. x+516. (Calcutta: Saha Institute of Nuclear Physics, 1957.) Rs. 25; 40*s*.; 6 dollars.

BY and large, there is only one thing wrong with this book—its date of publication. The reviewer's natural sympathy is with Prof. Saha, who completed the task interrupted by his father's death in 1956. But a manuscript completed in 1953 and only slightly revised before receipt in 1959 (although dated 1957), is not in keeping with the pace of physics to-day. The book is written primarily with the experimental scientist in mind; it covers relaxation time and moment measurements, quadrupole effects and chemical shifts in detail. Some mathematical ability and a knowledge of the language of quantum mechanics is assumed, but the arguments are presented in such detail that it should be easy for those learning the subject to follow them. In the authors' own words, "the attitude has been to discuss elaborately the phenomenological theory and the experimental techniques". They have achieved this aim, but it is of course the weakness of phenomenological theory that it covers only the cases discussed; extension and generalization are hazardous. The notable example of this difficulty is in the absence of a treatment of negative-spin temperatures.

The book is well printed, well indexed, and an errata sheet bound into the volume has corrected a good many minor slips. The bibliography is excellent up to 1956, and the book will save a good deal of searching of the literature before that date.

W. M. LOMER

The World is Round

The Story of Man and Maps. By Prof. Frank Debenham. Pp. 100. (London: Macdonald and Co. (Publishers), Ltd., in association with Rathbone Books, 1959.) 50*s*. net.

"THE World is Round," by Prof. F. Debenham, is "a far cry from those primitive attempts to the drawing of actual maps". The evolution of man's cartographical skill has been achieved by the use of vivid colour reproductions of imaginary world maps and ancient charts. Having traced man's global knowledge to the present day, the author uses specially designed colour relief maps to show the world as we know it to-day. Many unfamiliar sectors of the Earth are revealed and each continent is shown in relation to its neighbour, thus bringing out many points of comparison hitherto unrealized. Prof. Debenham avoids stress on one country more than another, and points out a sociological moral through a geographical fact; the Earth is indeed round, but its centre is not the place where we live.

The pages dealing with the historical background of each country are, in my opinion, out of place in a book of this kind, and tend to detract from its main purpose. From a concise section dealing with map-making instruments through the ages the reader is led to a review of man's latest inventions in mapping under the sea, under the land, and in outer space. This is a beautifully presented book that would not only provide a most useful aid in the school library but also would grace the shelf of any home.

M. CARADINE

La Vie des Colibris

Les Trochilidés. Par Ariane Martin et Anne Musy. (Les Beautés de la Nature—Série Exotique.) Pp. 246+32 planches. (Neuchatel: Éditions Delachaux et Niestlé, 1959.) n.p.

HUMMINGBIRDS, like butterflies and birds' eggs, are natural objects which interest the collector as well as the scientist; they are, as described in this book, "living precious stones" showing a fascinating variety of colour and form within the limits of their distinctive and homogeneous group. Although the object of the series to which this volume belongs is to extol the beauties of Nature, it is the biology rather than the art of hummingbirds which is the theme.

The text consists of a short historical note and chapters on geographical distribution, structure and morphology, temperature regulation, locomotion, food, reproduction and behaviour. These subjects are, of necessity, very briefly summarized. It is not widely known that hummingbirds have a temperature-control mechanism, so that in certain circumstances they can become torpid. Hibernation, in fact, was described in hummingbirds a long time ago.

To those who know little or nothing of these fascinating creatures this book conveys a fair summary of the facts of their biology. The text provides informative reading for the interested layman who, if he understands art, might also appreciate the colour plates.

J. D. MACDONALD

GEOMETRY OF THE STRUCTURE OF MONATOMIC LIQUIDS

By Prof. J. D. BERNAL, F.R.S.
Birkbeck College, University of London

SINCE the work referred to in my communication to *Nature* early in 1959[1], it has been possible to approach the problem in a more quantitative way by the use of computers and geometrical models. Following up the concept of a liquid as an essentially irregular assembly, I have been able to realize a mathematical model of such an assembly, thanks to the help of Dr. A. D. and Mrs. Booth at Birkbeck College, and of the University of London Computer Unit through the work of my son, Dr. M. J. M. Bernal. A method was devised for computing the co-ordinates of centres of random assemblies of hard spheres with the one condition that no two centres should approach at less than a minimum distance. It proved possible to arrange these in systems with varying densities, the chief interest being, however, in the arrangements of highest density. Later it was possible to build up such arrangements with as many as seventy-five spheres in various ways, to compute from these their mutual energies on the simple Lennard-Jones $-A/r^6 + B/r^{12}$ potential rule and also to determine their effective volumes.

The general picture which emerged was that each particular random arrangement could be altered in absolute scale so as to get a minimum energy for each mutual relative metric position of the points. It could further be modified without preserving these metrical relations but yet maintaining the same topological neighbourhood relations so as to get even lower energy values.

From these minimum energy arrangements it is possible to calculate mean radial distribution functions (Fig. 1). These resembled roughly those obtained experimentally by X-ray and neutron diffraction from monatomic liquids. Dr. Furukawa has shown[2] that nearly all metals show the same distribution function if these are calculated from their observed diffraction curves in the same way. The distribution functions derived from the random arrangement described are similar to this with the significant difference that the first or principal maximum is considerably broader in the model. Now this is exactly what should be expected from the way the model was derived by emphasizing minimum rather than most probable distances of neighbours, for it proved impracticable at this stage to devise a programme which ensured arrangements with a maximum of molecular contact.

To overcome this difficulty, I used a semi-empirical device by building an actual physical model corresponding to the machine-calculated distances. Then, after joining neighbouring points by links of variable length, devised by my technical assistant, Mr. J. Mason, I was able, by a process of manipulation, to reduce most, but by no means all, of the distances between neighbouring points to approximately identical minimal values. This gave a new kind of distribution function (Fig. 1) with much sharper peaks, sharper actually than those given by metallic or molecular liquids (Fig. 1) but more closely approximating to them than in the first model. It also gave a density of packing of 0·61, approaching the value of 0·63 determined by G. D. Scott (private communication) for irregularly close-packed spheres.

It is evident that this model, by an intermediate degree of equalization of the shorter bonds, could be made to provide radial distribution functions approximating, within experimental limits, to those calculated from diffraction curves. This agreement provides strong support for the principles of construction of the geometrical model proposed for liquid structure. Although, on account of the inherent phase ambiguity of diffraction studies, it is impossible to *prove* the correctness of the model by these means, it equally cannot be disproved, and it must correspond more closely to the real distribution than any model hitherto proposed. Its closest affinity is, in principle, to that of Furukawa[2], who was the first to explain the location of the second peak of the distribution function.

A contemplation of the bond-equalized model led me to the radically new conception of the *ideal structure of a liquid*, ideal in the sense that in such a structure all the distances between closest neighbours are equal. This ideal corresponds to the ideal structures in crystallography by which, for example, Pauling worked out the structures of the silicates. Actual structures, however, differ, and it is certain that no actual liquid structure can be an ideal one, though it may be close to it.

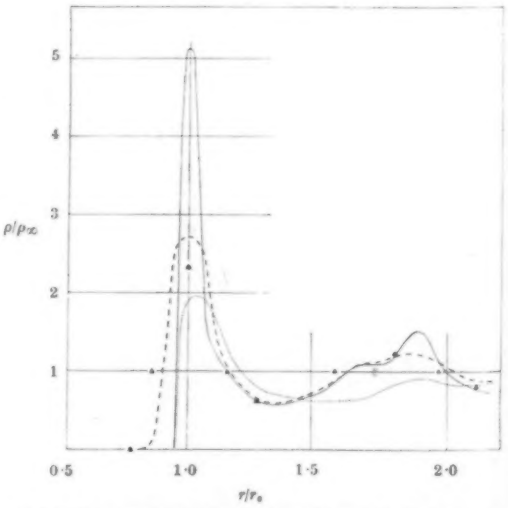

Fig. 1. Density distribution function: ρ/ρ_∞ variation of mean particle density as a function of r/r_0

. . . ., Derived from calculated random model; ——, derived from squeezed random model; - - - -, calculated for lead by Furukawa (ref. 2); ▲▲▲▲, calculated for liquid argon by neutron diffraction (Henshaw, D. G., *Phys. Rev.*, **105**, 976; 1957)

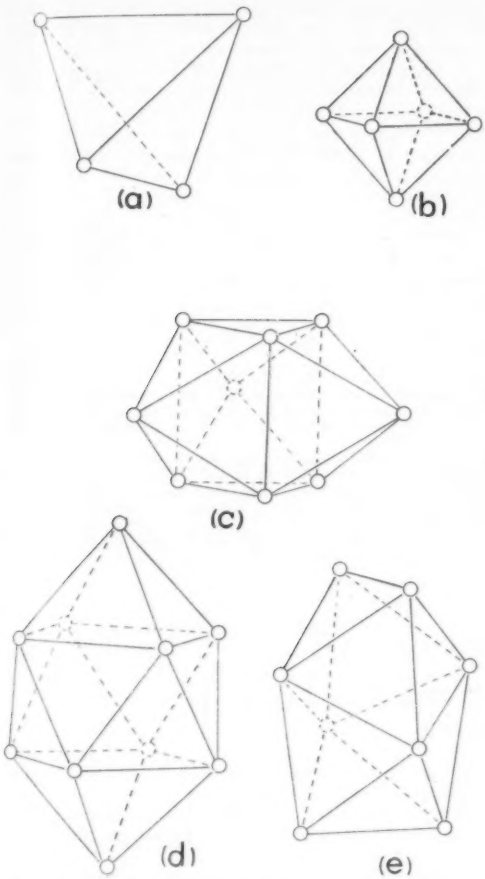

Fig. 2. Forms of holes in ideal equidistant molecule model of a liquid. *a*, Tetrahedron; *b*, octahedron; *c*, trigonal prism capped with three half octahedra; *d*, archimedean antiprism capped with two half octahedra; *e*, tetragonal dodecahedron. (Note prevalence of five-fold surface co-ordination in *c*, *d* and *e*)

Examination of the model showed that it could be considered most profitably as broken down into a set of *polyhedral* holes between molecules. These polyhedra are necessarily small because, by definition, in any coherent *close-packed* liquid there are no holes large enough for another molecule to be inserted. All have in common faces composed of equilateral triangles, or, in other words, *all their edges are equal*. They can, in fact, be derived from five ideal basic shapes: two platonic, two regular archimedean polyhedra, and one of lower symmetry. These are the tetrahedron, the octahedron, the trigonal prism, the archimedean antiprism, and another eight-cornered form, the tetragonal dodecahedron (Fig. 2). These forms can be combined in an unlimited number of ways through the sharing of triangular or square faces (here the half octahedron can appear as a pyramid on a square base). There are no other forms with equal edges, though a relaxation of this rule allows a few other kinds of holes to occur. However, *in general*, such packing cannot fill space without breaking, though to a degree limited to some 15 per cent, the condition of the equality of nearest neighbour distances. The introduction of archimedean polyhedra prevents any long-range order and also prevents a realization of the ideal structure precisely; for the sums of the dihedral angles of the polyhedra can never (except in the case mentioned below) be made exactly to amount to four right angles. In one particular case, that where only tetrahedra and octahedra occur, the packing can be regular, giving rise to varieties of cubic or hexagonal close-packed crystals.

It is possible, therefore, to formulate the geometrical hypothesis of the structure of liquids as follows:

In an *ideal* liquid structure, the molecular centres lie at the apices of a set of empty polyhedra (holes) with equal edges (r_0), or a combination of them. These polyhedra are: platonic regular (1) tetrahedra; (2) octahedra; archimedean (3) trigonal prisms; (4) archimedean antiprisms; and (5) tetragonal dodecahedra. The presence of the three latter forms prevents any long-range order and thus permits fluidity appropriate to temperature, while their proportion determines the configurational volume of the structure at different temperatures. For any temperature the structure is characterized only by the mean value $\overline{N}$ of molecular neighbours, varying from 11 to 3 as the temperature rises, and by the variance (λ) of the intermolecular distance (r_a), which increases with temperature.

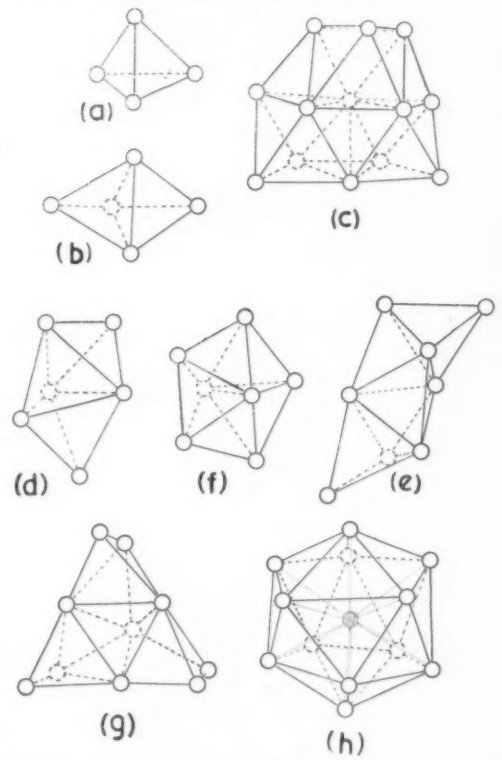

Fig. 3. Crystal nuclei and pseudo-nuclei built from equidistant or quasi-equidistant molecules. Crystal nuclei: *a*, single; *b*, double tetrahedra; *c*, close-packed layers, four tetrahedra and three octahedra. Pseudo nuclei: *d*, three; *e*, four tetrahedral elements of super close-packed spiral chain; *f*, five tetrahedra in pentagonal bipyramid; *g*, seven tetrahedra with elements of three spiral chains; *h*, twenty tetrahedra as fitted in an icosahedron. (In *f* and *h* all the intermolecular distances cannot be precisely equal)

This concept of the ideal structure also opens the way to a real statistic of liquid structure, the relative numbers of the platonic and archimedean polyhedra, depending on the temperature, giving the possibility of determining a mixing entropy to account for the large entropy of the liquid state. This would seem to provide a geometrical basis for Eyring's[3] partition function in his new thermodynamic theory of liquid structure. It can be seen that this geometric view contains the essential features of many earlier theories of liquids, including that of Born and Green[4] based on gas laws, the hole theory of Kirkwood[5] and Frenkel[6] and the crystallite theory of Eyring and others, and points to their eventual reconciliation.

With regard to the last of these, it is apparent from the model that even the most irregular structure contains regions composed of a number of tetrahedra sharing faces. Even though their density may be greater than close packing, as Boerdijk[7] has shown, they are not in general crystalline, but are asymmetric or have forbidden symmetries, such as pentagonal (Fig. 3). They cannot lead to three-dimensional repeat structures and consequently cannot function during the time of their stability as nuclei of crystallization. This may indeed provide the explanation of the phenomena of nucleation and crystal growth in super-cooled liquids. The character of these pseudo-nuclei near their melting point may well be dependent on whether they are formed from the melting of a crystal or from the cooling of a liquid heated to a high temperature. This may also explain some observed hysteresis phenomena in liquids near the melting point. It also implies that with poly-atomic molecules there may be several degrees of melting or break-up of such non-crystallographic aggregates which may help to account for viscosity anomalies with temperature which will appear not as discontinuities but changes of higher order. Further, the existence of such condensations indicates the necessity of fluctuations in density in normal liquids of the dimensions of several interatomic distances as postulated by Furth[8].

A fuller account of this work will be published elsewhere.

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SALTS AND RESPIRATION

By Prof. H. LUNDEGÅRDH
Penningby, Sweden

ANIONS and cations of inorganic salts are non-metabolically absorbed by the protoplasm, a process obeying common principles of ion exchange and Donnan equilibrium[1], but metabolic energy is always needed for accumulation of these salts in the vacuoles[2,3]. Even if the initial salt absorption of the protoplasm is a spontaneous process, it is important to realize that the power of salt absorption of the protoplasm, namely, the amount of ion carriers (symbolized as R^- and R^+), is also metabolically regulated[4]. Little is known about the chemical nature of the carriers. They behave as large organic ions and reflect the ionic activity of the protoplasm itself. Normal aerobic respiration is needed for maintenance of the structural qualities of the protoplasm. The activity of the ion carriers is concommittantly more or less abolished if the cells are poisoned by cyanide[4].

From a biochemical point of view the pronounced sensitivity to cyanide points to co-operation of the cytochrome system in the absorption and accumulation of salts. This conclusion had been drawn in 1935[2a]. Later on, direct observations of the response of the respiratory enzymes to presence or absence of salts were made[5]. Rapid measurements of the height of absorption bands of reduced cytochromes a_3, a, c and b of a bundle of wheat roots showed that the bands are high if the roots are 'desalted', that is, thoroughly washed in distilled water, and decrease in height upon addition of 0·001–0·005 M solutions of neutral salts or dissociated salts of organic acids (see Fig. 2). Recent results show a similar response of diphosphopyridine nucleotide and flavoprotein, which are also participating in the respiratory chain. The response to salts is thus confined to the *total* respiratory system, not only to single enzymes.

Discussions of the mechanism of salt accumulation are frequently hampered by ignorance of this distinction between 'initial absorption' and 'continued accumulation'[4,6]. A large number of cells, among them most animal cells and meristematic cells of plants, have much less 'free water' than fully grown cells of plants, in which the sap space may occupy more than 95 per cent of the total volume. The process of continued accumulation is consequently much more dominating in the latter. A question of considerable theoretical interest was raised by recent observations of a sudden stimulation of aerobic respiration upon addition of salts, namely, stimulation occurring in the initial period before the full start of continued accumulation[4]. Because in plant tissues the two phases of absorption are fairly close together in time (the phase of accumulation starts about 15 min. later than the initial absorption), a closer study of the problem required an organism in which salt accumulation takes a subordinate role. Baker's yeast is such an organism. Most inorganic salts pass into the yeast cell, but no significant accumulation above the level in the medium was observed. Yeast is provided with a complete cytochrome system which, in co-operation with pyridine nucleotide and flavoprotein, maintains a fairly intensive aerobic respiration.

The Salt Effect on Baker's Yeast

Suspensions (5–15 per cent) of washed baker's yeast were investigated in the Barcroft–Warburg

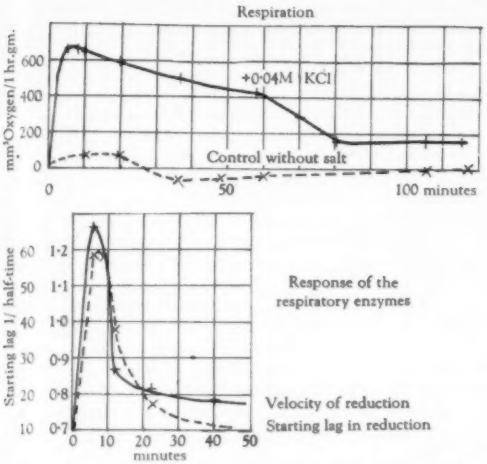

Fig. 1. Response of oxygen consumption (above) and activity of the respiratory enzymes to an addition of 0·04 *M* potassium chloride to a suspension of washed baker's yeast. Identical vessels were investigated, one set without and one with salt. The initial rapid burst of respiration is recognized both in oxygen consumption and reaction of the enzymes

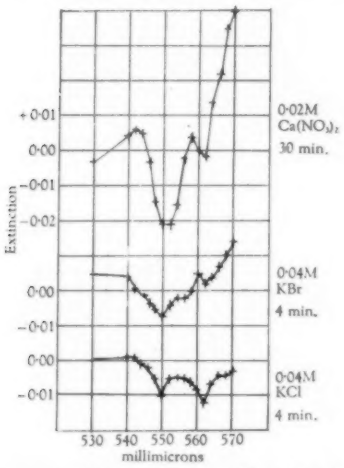

Fig. 2. Response of the cytochromes *c* (550 mμ) and *b* (562 mμ) to an addition of salts. Difference spectra were recorded, one quartz vessel (see ref. 7) containing yeast without salt, the other with salt. 1 per cent glucose was added to both vessels in order to obtain 90 per cent reduction from the start. The response on salts implies lowering of the reduced peaks

respirometer and simultaneously in recording spectrophotometers of my own construction[7]. The spectrophotometric observations were carried out according to the procedure developed in my laboratory for studies of the kinetics of the respiratory enzymes, namely, difference spectra were recorded between control (in water) and sample (with salt). Records were made during consecutive time-intervals. For the determination of start lags and apparent reaction velocities of reduction and oxidation, intermittent gassing with nitrogen or air respectively was used, according to a previously elaborated technique[8]. The spectra were in this case recorded by means of a rapid-scanning spectrophotometer.

The response of the oxygen consumption to addition of salts is shown in Table 1 and Fig. 1. Figs. 2, 3 and 4 show the corresponding response of the enzymes.

Table 1. Increase of Respiration on Addition of Salts. 2 c.c. 15 per cent Yeast Suspension in each Warburg Vessel. Salts added up to 0·05–0·20 *N*. Time of Experiment, 110 min. Relative Values, Control without Salt = 100

(*A*) *Series with start in distilled water*

I. Addition of potassium salts						
Anion :	Cl	Br	I	NO_3	HPO_4	SO_4
	121	122	117	117	127	117
II. Addition of nitrate						
Cation :		K		Ca		Mg
		117		141		140
III. Addition of chloride						
Cation :		K		Ca		NH_4
		121		115		116

(*B*) *Series in* 1 *per cent glucose*

I. Addition of potassium salt						
Anion :	Cl	Br	I	NO_3	HPO_4	SO_4
	126	131	133	128	129	124
II. Addition of nitrate						
Cation :		K		Ca		Mg
		127		129		128
III. Addition of chloride						
Cation :		K		Ca		Na
		126		144		141
IV. Addition of sulphate						
Cation :		K		Mg		
		126		123		

Table 1 shows that salts increase the respiration 20–30 per cent both in distilled water and in 1 per cent glucose, and that this stimulation is fairly unspecific, primarily with respect to different anions. The response to cations is comparatively unspecific, too, with the exception of magnesium and calcium, which are known to interfere with biochemical processes.

As shown by Figs. 1, 3 and 4, the sudden burst of respiration, starting 1–2 min. after the addition of salt (concentrations of 0·01 up to 0·2 *M* were tested), depends upon a corresponding increase in the activity of the enzyme system. Cytochrome *c* as well as diphosphopyridine nucleotide show a sudden increase of the velocity of reduction in nitrogen and re-oxidation in air (measured as the reciprocal of half-time). Similar observations were made regarding cytochromes a_3, *a* and *b*, of which a_3 responds very rapidly. The lag at starting, that is, the time elapsing from switching on nitrogen to the start of

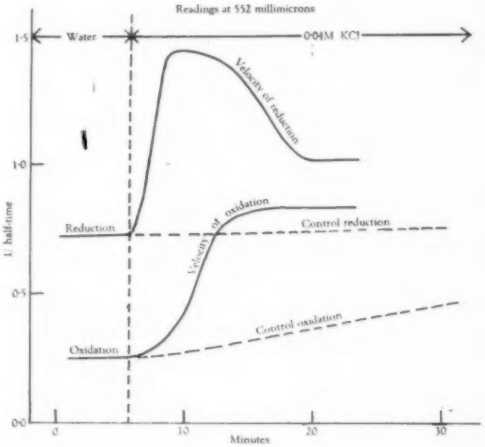

Fig. 3. Response of the velocities of reduction and re-oxidation to the addition of salt. Control and sample were both exposed to alternate gassing with nitrogen and air at constant intervals (see ref. 8) and the response at 552 mμ (cytochromes $a + c_1$) continuously recorded

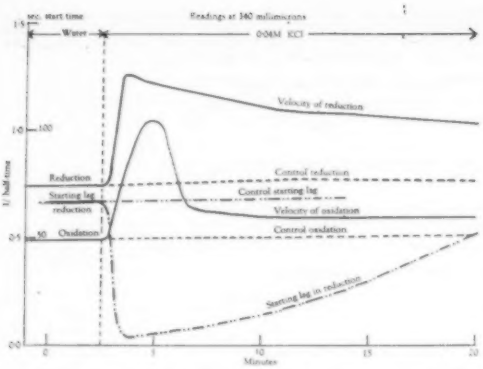

Fig. 4. Response of the velocities of reduction and re-oxidation and of the starting lag in reduction to an addition of salt. As in Fig. 3, but diphosphopyridine nucleotide at 340 mμ recorded

reduction (see ref. 8), drops considerably, too, a fact reflecting the rise in oxygen consumption; the dissolved oxygen disappears more rapidly if the cytochromes are activated.

It will be seen from Figs. 1, 3 and 4 that the sudden activation of the respiratory system attains an optimum a few minutes from the start and then slowly declines, even if the values still remain higher than in the controls. This fact may be explained as follows: the salt effect implies an acceleration of electrons through the respiratory system; this means a sudden over-consumption of substrate until a new balance between internal supply of substrate and oxygen consumption is attained.

Principle of Anion Respiration

The new experiments corroborate earlier results as to a non-specific salt (anion) effect[2–4]. This fact, as well as the observation that all enzymes in the respiratory chain are equally affected, is evidence against a closer combination between salt ions and enzymes. The new results are in full agreement with the principle of electrolytic regulation of the velocity of electron transference in the respiratory chain[2b]. It was originally assumed that electrons and anions are exchanged between single enzyme molecules (see below). This idea is still valid, but more recent results point to the existence of a spatially organized respiratory unit displaying electro-chemical properties similar to those of a galvanic cell.

Biochemical investigations are mostly confined to more or less purified and 'dissolved' enzymes, the reactions of which are proceeding in Newtonian media and obeying simple thermodynamic principles. It is, however, very unlikely that enzymes in the live protoplasm are behaving similarly to 'dissolved' enzymes. The regulated co-operation of numerous enzymes in the protoplasm points to the existence of a complicated spatial organization, in which the single enzymes occupy distinct areas. The activity of such enzymes will be guided by the spatial orientation of the molecules. It may be justified to speak of spatial biochemistry. The frequently used term 'multi-enzymes'[9] does not quite cover the conception of spatial organization, because important building stones of an enzyme system are 'factors' such as lipides, quinones, certain vitamins, etc. These factors serve as pathways or switchboards regulating the passage of electrons and intermediates between the reaction centres.

Little is known yet of reactions going on in organized enzyme systems, but it is possible to trace indirectly the existence of a spatial organization.

Observations of rhythmical pulsations in the stream of electrons through the respiratory system may be interpreted as a reflexion of its spatial organization[8,10]. Observations on homogenates of tissues point in the same direction. It was shown that even a high degree of mechanical disintegration of plant protoplasts does not change the quantitative relations between cytochromes a_3 and b. Spatially organized 'respiratory units' apparently compose the sub-microskeleton of the protoplasm, and it is reasonable to assume that these units are joined to microscopic structures, for example, surface layers of 'mitochondria'[1,2,4], the tonoplast of vacuolized cells, etc. We are now prepared to discuss the main problem, namely, the electrochemical properties of the respiratory unit.

According to recent observations[8,10], the respiratory system is composed of three spatially arranged groups of enzymes, namely: (1) enzymes linked with diphosphopyridine nucleotide; (2) flavoproteins and cytochrome b; and (3) cytochromes c and a_3. A number of accessory cytochromes (a, c_1, b_3, etc.) and 'factors' are additional elements of which some probably trap energy as high-energy phosphates. We know that diphosphopyridine receives electrons and protons from metabolites (*via* the tricarboxylic acid cycle) and transfers the electrons to the group flavoproteins + cytochrome b, which in its turn shuffles electrons to the group cytochromes $c + a_3$. From cytochrome a_3 the electrons leave the system with oxygen as the final acceptor.

The extremely rapid re-oxidation of a reduced respiratory unit indicates a location of the 'pole of oxidation' (o), namely cytochrome a_3, close to the surface of respiratory particles or membranes, whereas the 'pole of reduction' (i) namely, diphosphopyridine nucleotide, etc., is probably turned in a more or less opposite direction. As shown by Fig. 5, a stream of electrons moving from the pole of oxidation to the pole of reduction will then act as the internal circuit of a galvanic battery.

In the absence of an outer circuit, no electrons flow through a galvanic cell. A current starts, however, if the cell is surrounded by a salt solution, because the electrophoretic movement of ions in the outer circuit is a measure of the velocity of internal flux of electrons. The intensity of the current is regulated by the concentration (activity) of the salt ions.

This scheme may be applied to the conditions prevailing in the living cell. In the absence of salts, the stream of electrons from cytochrome a_3 to the dissolved oxygen molecules is slowed down to a minimum and the level of reduction of the respiratory enzymes rises. Because hydrogen ions and hydroxyl ions, together with anions of organic acids, are produced by the activity of the system itself, some 'respiratory idling' still proceeds; but observations on plant tissues and yeast show that lack of salts cuts down respiration to at least 50–75 per cent. Renewed supply of salts rapidly speeds up the stream of electrons. Because the capacity of dehydrogenation at the pole of reduction (Fig. 5) is less susceptible to changes, the immediate effect is a rising oxidation of the respiratory enzymes.

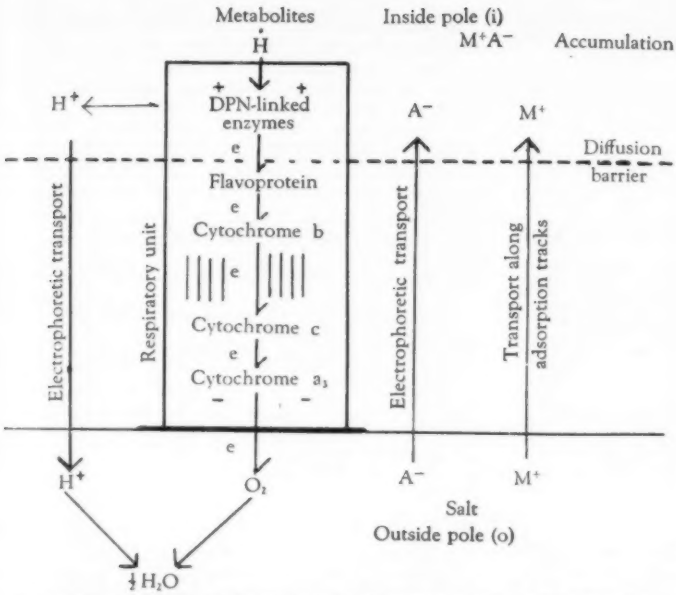

Fig. 5. Scheme of the principle of salt respiration. The respiratory unit is shown operating between surroundings containing free salts and an internal space behind a barrier which slows down the passive flow back of accumulated salts. The scheme may be applied to salt accumulation in the vacuoles of plant cells or in the interior of mitochondria. The initial salt absorption to carriers is omitted in this scheme (see text)

Biological Importance of Anion Respiration

The principle of anion respiration sheds light on a number of biological phenomena. It is a well-known fact that organisms produce electricity, even if only few of them, for example, the electric ray, store high-voltage static electricity. A circuit may be closed between the leaves and the roots of a plant and currents are also produced by root tips 1–2 cm. long[2b]. The respiratory character of the current is shown by the fact that the respiration rises or falls if the normal current is increased or diminished by means of an external supply of electricity. It may be expected that electric shocks directly affect the power of respiration, even if severe disturbances in the assembly of cations, which are adsorbed to cell structures, may be responsible for more 'explosive' effects.

Cytochrome respiration is a common condition for normal life and reproduction of cells, a thesis valid for almost all organisms—animals, plants and micro-organisms. A reflexion of the synthetic activity is the regulation of the amount of ion carriers referred to above. Malignant cells, for example, in cancer tumours, on the other hand, show a pronounced increase of glycolytic systems at the expense of cytochrome respiration[12]. Owing to stimulation of the cytochrome respiration, salts would perhaps help in curing tumours or mitigate deleterious effects of irradiation and/or to some extent protect normal cells from being affected by cancerogenous substances or irradiation. These are still unsolved problems. Deficiency in salts is a well-known cause of pronounced disturbances in the normal function of the animal organism.

Because transference of electrons occurs also outside of the scope of the 'respiratory unit' and because cytochromes may turn over electrons to acceptors other than oxygen, for example, in certain bacteria and in photosynthesis[15], salts may be able to display non-specific effects on many processes. An example of switching over glycolytic processes to a more efficient anaerobic utilization of glucose is given by wheat roots. In absence of oxygen, desalted roots show a 'glucose co-efficient' (consumed glucose/carbon dioxide produced) of 0·5. Addition of salt lowers the coefficient to values of about 0·33 or less.

The unspecific salt effect refers primarily to organized enzyme systems which are transferring electrons. It is, however, evident that a transference of electrons between two 'dissolved' compounds is also facilitated by salts. Salt anions will in this case probably be directly shuffled against electrons according to the scheme:

$$\text{reduced substance} + A^- \rightarrow (\text{oxidized substance}).A^- + e$$

provided that e is caught by an acceptor. Such a salt stimulation has been observed with regard to oxidation of cytochrome *c* (ref. 14).

According to Fig. 5 organized systems induce streaming of anions from the negative (*o*) to the positive (*i*) pole and of cations in the reversed direction. Because cations (H^+) are produced by the respiratory unit itself, but not anions, the regulating factor is primarily the supply of anions, hence the conception 'anion respiration'. If no semipermeable boundaries interfere, diffusion and mass streaming will perpetually counteract any appreciable accumulation of ions at the poles. The response of the respiratory units to salts will thus induce stirring in the surroundings and an indirect acceleration of the mixing of substances.

The synthesis of ion carriers is probably linked to the trapping of respiratory energy in products of phosphorylation[4], but very little is known about the chemical nature of these processes. The fact that cytochrome respiration maintains and reinforces the ability of the protoplasm to attract and retain salts is obviously of fundamental importance to the life of the cell.

The accumulation of free, that is, non-adsorbed, salts in the sap spaces of plant cells must be regarded as a special process implying the active transference of initially absorbed salts to the vacuoles. The retention of free salts is obviously caused by the operation of a barrier which precludes the rapid back-flow of ions once accumulated. Fig. 5 illustrates the effect of a diffusion barrier placed between the poles of the respiratory unit. A monolayer of respiratory units will thus be able to accumulate anions. These anions will combine spontaneously with cations which are adsorbed to the protoplasm, as a consequence of which free salts are accumulated (see Fig. 5). It is feasible to assume that the vacuole membrance (tonoplast) acts in some such way. The fairly constant values of anion/oxygen observed in

plant tissues point in this direction[2,4]. Accumulation of free salts would, of course, also occur in 'mitochondria' if the respiratory units are arranged in a monolayer coating the particles. Little is known, however, of true accumulation of salts in the 'free water' of protoplasts which do not contain vacuoles.

The investigations have been supported financially by the Swedish Research Councils of Natural Science and Agriculture.

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NEWS and VIEWS

New Year Honours List

The following names of scientists and others associated with scientific work appear in the New Year Honours list:

Baron: Sir George Nelson, chairman of the English Electric Co., Ltd.

Baronet: Sir James Paterson Ross, president of the Royal College of Surgeons of England.

K.C.M.G.: Sir Charles Blackburn, chancellor of the University of Sydney, for services to education and medicine; Dr. George A. Currie, vice-chancellor of the University of New Zealand; Sir Philip Morris, lately chairman of the Commonwealth Education Conference, vice-chancellor of the University of Bristol; Geoffrey W. Nye, adviser on agriculture to the Secretary of State for the Colonies.

Knights: Prof. Edward Ford, director of the School of Public Health and Tropical Medicine, University of Sydney; Dr. William H. Glanville, director of the Road Research Laboratory (Department of Scientific and Industrial Research); Dr. John Hammond, formerly reader in agricultural physiology in the University of Cambridge; William W. S. Johnston, of the State of Victoria, for his work for postgraduate education in medicine; Prof. Harrie S. W. Massey, Quain professor of physics, University College, London.

From January 1960, issues of *NATURE* will be grouped into four volumes a year, instead of two as heretofore

C.B.: M. I. Michaels, under-secretary, Office of the Minister for Science; R. N. Quirk, under-secretary, Office of the Minister for Science; R. H. Weir, director-general of engine research and development, Ministry of Aviation; Dr. H. F. Willis, chief of the Royal Naval Scientific Service.

C.M.G.: A. W. Knight, chief commissioner of the Hydro-Electric Commission, State of Tasmania; R. E. Stedman, under-secretary, Ministry of Agriculture, Fisheries and Food; C. W. Michie, permanent secretary, Ministry of Agriculture, Northern Region, Nigeria; S. E. Morris, secretary for native affairs and chief native commissioner, Southern Rhodesia; Dr. E. W. Russell, director of the East African Agriculture and Forestry Research Organization.

C.B.E.: Prof. S. Alstead, regius professor of materia medica and therapeutics, University of Glasgow; H. E. Bannister, assistant secretary, Ministry of Agriculture, Fisheries and Food; J. D. Clark, director of the Rhodes–Livingstone Museum, Northern Rhodesia; C. E. Clarke, principal actuary, Government Actuary's Department; Dr. T. F. Davey, senior specialist (leprologist), Eastern Region, Nigeria; L. J. Davies, director of research and education, British Thomson-Houston Co., Ltd.; J. Edwards, head of Production Division and scientific adviser, Milk Marketing Board; Dr. A. J. P. Martin, for services in the development of chromatographic analysis; Dr. D. C. Martin, assistant secretary of the Royal Society; Dr. A. T. R. Mattick, lately deputy chief scientific officer, National Institute for Research in Dairying, Shinfield, Reading; T. B. Nicol, engineer-in-chief, Metropolitan Water, Sewerage and Drainage Board, New South Wales; H. T. Pledge, keeper in the Science Museum, South Kensington; W. T. Price, principal of Harper Adams Agricultural College, Newport, Shropshire; H. T. Ramsay, director of the Safety in Mines Research Establishment, Ministry of Power; J. B. Randall, director of Veterinary Services and Animal Industry, Uganda; F. I. G. Rawlins, deputy keeper and scientific adviser, National Gallery; G. B. Ryle, director of forestry for England, Forestry Commission; Prof. R. W. Scarff, professor of pathology, University of London; V. C. Williams, of Griffith, New South Wales, for services to irrigation research.

Royal Society Vice-Presidents

The president of the Royal Society, Sir Cyril Hinshelwood, has appointed the following vice-presidents for the year ending November 30, 1960: Sir William Penney, treasurer of the Royal Society, member for scientific research, United Kingdom

Atomic Energy Authority; Sir Lindor Brown, biological secretary of the Royal Society, Jodrell professor of physiology at University College, London; Sir William Hodge, physical secretary of the Royal Society, Lowndean professor of astronomy and geometry in the University of Cambridge; Dr. H. G. Thornton, foreign secretary of the Royal Society, lately head of the Department of Soil Microbiology at Rothamsted Experimental Station; Sir Patrick Linstead, rector of the Imperial College of Science and Technology; and Prof. Wilson Smith, professor of bacteriology at University College Hospital Medical School in the University of London.

Biology at Middlesex Hospital Medical School: Prof. J. H. Woodger

Prof. J. H. Woodger has just retired from the Middlesex Hospital Medical School after thirty-seven years as head of the Department of Biology. He graduated at University College, London, in 1914 and returned as assistant in zoology after four years active service, mainly in Mesopotamia, before accepting an appointment in the Middlesex Hospital Medical School at the age of twenty-eight. At a very early stage in his career, Woodger discerned that much of what passed for 'zoological philosophy' was a hotch-potch of confused thinking based upon an inattentive or insufficiently analytical use of words. In his "Biological Principles" of 1929 he did his utmost to dissect some of the stock antitheses of biology—for example, the antithesis between preformation and epigenesis—with the resources of ordinary language; but ordinary language proved too clumsy and imprecise, and Woodger's thoughts accordingly turned to the logical symbolism of Russell and Whitehead's "Principia Mathematica", enriched by the addition of many empirical constants for ideas which (unlike number or similarity) cannot be defined by logical concepts alone. His "Axiomatic Method in Biology" of 1937 was the first attempt to 'axiomatize' biology, that is, to cast it in the form of theorems logically deducible from a small number of axioms or postulates. Woodger has an international reputation as a logician; his "Technique of Theory Construction" of 1939 is a splendid exposition of the modern ideas on scientific methodology that arose mainly from the work of Karl Popper. Woodger's fine analytical work was continued in his Tarner Lectures of 1949–50 and is still actively in progress. His attempts at axiomatization have been thought premature by some who are competent to express an opinion; but the attempt to import rigour and precision into biological thinking was very much overdue, and Woodger's writing and thinking have had a quiet but deep and pervasive influence over a great deal of modern biological thought. Woodger stands in the mainstream of the great tradition of British empiricism; and his work marks the final repudiation of the metaphysical elements in *Naturphilosophie*.

Prof. D. R. Newth

Prof. Woodger's successor, Dr. D. R. Newth, is also a graduate of University College, London; and, like Woodger, he spent four years on active service between graduating in zoology in 1942 and returning to postgraduate work in 1946. Prof. Newth is an embryologist and the son of an embryologist; but narrow sectarian classifications are no longer appropriate in biology, and he is best described as a biologist of unusually wide knowledge and sympathies whose main concern has been with the biological problems raised by embryogenesis. His first work was on the normal development of amphibian embryos. He then turned to the largely neglected problems raised by the evolutionary history of the neural crest, and undertook an experimental analysis of its behaviour and fate in lampreys. After an interlude in which he worked upon the reactions of hagfishes to light, he returned to morphogenetic problems. His was the first rigorous attempt to answer the question: Can an animal 'regenerate' a part which it never possessed? In certain circumstances, he found, it can do so: by interfering with the primary embryogenesis of structures which are capable of regeneration in later life, they may develop abnormally; but these abnormalities can be partially made good if the abnormal part is amputated and so caused to grow anew. His analysis provides one possible approach to the problem of the persistence of post-embryonic life of the available genetic information in somatic cells. Newth's appointment will give particular pleasure not only to biologists in Great Britain but also to his many colleagues on the editorial board of the international *Journal of Embryology and Experimental Morphology*, in the foundation of which he was one of the prime movers.

Forest Products Research Laboratory

The Council for Scientific and Industrial Research has reviewed the operation of the arrangements made early in 1958, under which it asked the Timber Development Association to assume responsibility for certain aspects of timber research previously discharged by the Forest Products Research Laboratory. The Steering Committee for the Laboratory had become convinced that the new arrangements did not allow sufficient latitude to the Laboratory in the discharge of its essential duties, and that the division of functions between the Laboratory and any other research organization must be more flexible. It was sure, therefore, of the need for a new approach to the problem of securing industrial support of work done for the immediate benefit of the timber trade (which was the aim of the earlier arrangements) and also to provide for the needs of the users—the home producer (including the Forestry Commission), the merchant and the manufacturer. At the same time it was regarded as essential to maintain an adequate and well-balanced research team at the Forest Products Research Laboratory. The Research Council has therefore re-stated its general policy for the Forest Products Research Laboratory in the following terms: "There is a continuing need in the national interest, both for research of a fundamental character on timber (but with ultimate practical applications) and for work of immediate benefit to the Forestry Commission and timber users. This work is best done at the Forest Products Research Laboratory with financial support from industry wherever possible. The Laboratory's programme should be co-ordinated with that of the Timber Development Association Research and Development Committee and/or of any Research Association which may be set up, but should primarily be devised to ensure that the interests of Government and the whole timber industry (producers and users, as well as merchants) are served to the best advantage".

Graphite Blocks for the Dounreay Reactor

The Morgan Crucible Co., Ltd., of Battersea, London, S.W.11, has now been permitted to reveal that for the first time special borated graphite blocks were used by the United Kingdom Atomic Energy Authority in the assembly of the shield which surrounds the reactor vessel at the Authority's experimental station at Dounreay. The blocks were made and machined by the Morgan Crucible Co., at its works at Norton, near Worcester. This factory is not engaged in the production of nuclear pure material and was therefore chosen as one suitable for handling large quantities of boron compounds. Most of the borated graphite contained 0·3 per cent boron, but some contained 5 per cent—in both cases very uniformly distributed throughout the graphite. The blocks themselves were machined to five hundred different patterns, some of which were so intricate that wooden dummies were made to ensure that it was practicable to machine them in graphite. As a final check, the layers of finished graphite blocks were assembled at Norton. The blocks, painted with distinguishing coloured bands to avoid any risk of confusion with nuclear pure material, were then packed into cartons for the journey. Transport to Dounreay was by heavy lorry, which made one round trip of 1,500 miles per week, taking the whole dry season of one year—some eight months in all.

World Meteorological Organization Conferences and Symposia, 1960

The World Meteorological Organization has issued a provisional schedule of conferences and symposia to be organized in 1960: *January*, Meeting on Atomic Transport and Removal of Radioactive Debris from Nuclear Tests (New York); *March*, Facsimile Broadcasts of the Working Group on Telecommunications, established by Regional Association VI (Offenbach–Frankfurt); *April*, Eastern Caribbean Committee of Regional Association IV of North and Central America (Curaçao); *June* 27–*July* 15, Executive Committee—twelfth session; *August*, Working Group on Telecommunications of the Commission for Synoptic Meteorology; *August* 16–*September* 3, Commission for Maritime Meteorology—third session (Utrecht); *September–October*, Regional Association VI, Europe—third session (Lisbon or Madrid); South American Regional Seminar on Tropical Agrometeorology (Venezuela); Working Group on the Guide to Climatological Practice established by the Commission for Climatology (Geneva); *November* 21–26, Working Group on Telecommunications—established by Regional Association I, Africa—fifth session (Cairo); *November* 28–*December* 16, Regional Association I, Africa—third session (Cairo); *December* 1–16, Commission for Climatology—third session (London).

Recent Geophysical Work in Great Britain

The meeting of the Geological Society of London on January 27 will take the form of a symposium on "Geological Implications of Recent Geophysical Work in the British Isles". The afternoon session will discuss "The Study of Igneous Rock Masses". The principal speakers will be Dr. M. H. P. Bott (University of Durham), who will speak on granites, crustal structure and isostasy, and Dr. D. J. Blundell (University of Birmingham), whose subject will be the use of rock magnetism in studies of igneous geology. Other contributors will be Prof. T. Murphy (School of Cosmic Physics, Dublin) and Mr. S. H. U. Bowie (chief geologist, Atomic Energy Division, Geological Survey). The evening session will consider "The Study of Sedimentary Basins". The principal speakers at this session will be Mr. N. G. O'Halloran and Mr. R. G. W. Brunstrom (British Petroleum Co.), on the use of seismic reflexion methods in geophysical surveying, and Dr. W. Bullerwell (Geological Survey), on aeromagnetic surveys over central, eastern and southern England. Prof. W. B. R. King and Mr. G. Armstrong (chief geologist, National Coal Board) will also speak. At the close of the evening session the discussions at both sessions will be summarized and reviewed by Prof. J. H. Taylor (King's College, London).

"Inadequacy of Scatter Mechanisms in Tropospheric Radio Propagation"

In a communication under this title in *Nature* of November 14, p. 1558, it was stated that "the lenses have an average size of a few kilometres diameter". Mr. P. C. M. de Belatini states that this should read "a few metres". The toroids themselves are believed to be on average about 600 metres in diameter with an 'eye' that acts as the lens of about 15 metres diameter. These dimensions, of course, vary with time.

Announcements

Mr. B. A. Worswick, recently chief engineer of Fischer and Porter, Ltd., has been appointed technical director with a seat on the Board of Elcontrol, Ltd. He was previously chief development engineer of Bailey Meters and Controls, Ltd., after having served for some years with Imperial Chemical Industries, Ltd.

The International Sericultural Commission has announced that the second International Technical Sericultural Conference will be held in Murcia, Spain, during April 2–5. Further information can be obtained from A. Schank, General Secretary-founder, Commission Séricicole Internationale, Station de Recherches Séricicoles, 28 Quai Boissier de Sauvages, Alès, France.

The Brighton Technical College School of Pharmacy has announced that a course of eight evening lectures on Spectroscopic Methods of Pharmaceutical Analysis will be held during the period February 9–March 29, at the Main Hall of the College, Richmond Terrace, Brighton 7. Further details can be obtained from Dr. J. C. Parkinson, School of Pharmacy, Technical College, Brighton 7.

A conference on the Actinomycins and their Importance in the Treatment of Tumours in Animals and Man will be held under the auspices of the New York Academy of Sciences at the Barbizon-Plaza Hotel in New York City during March 31–April 1. Further information can be obtained from the Executive Director, New York Academy of Sciences, 2 East 63rd Street, New York 21, N.Y.

Errata. In the communication entitled "A Functional Interpretation of the Electron-Microscopic Structure of the Sensory Hairs in the Cristæ of the Elasmobranch *Raja clavata* in Terms of Directional Sensitivity" in *Nature* of December 5, p. 1807, Prof. O. Lowenstein points out that there are two mistakes: par. 3, line 1, for "when" read "why"; a grant acknowledged in the penultimate paragraph was from the Swedish Medical Research Council, not the Karolinska Institutet as printed.

CORNELL UNIVERSITY CENTER FOR RADIOPHYSICS AND SPACE RESEARCH

A NEW research centre is to be set up by Cornell University, Ithaca, New York, for the purpose of conducting research, and training research workers, in the subjects of radiophysics and space research. The centre will operate a high-powered radar equipment in Puerto Rico, financed by the Department of Defense. This equipment will make use of a fixed parabolic mirror with a diameter of 1,000 ft. built into a natural hollow in the mountains. It will have a peak power of 2·5 MW. In addition, it is proposed that there should be a radio-astronomy receiver near Ithaca, and a transmitter on the campus of Cornell.

The director of the new Centre will be Prof. T. Gold, who will be assisted by Prof. H. G. Booker as associate director. Both Gold and Booker went to the United States from Great Britain. Both worked on radar in England during the Second World War, Gold at the Admiralty Research Establishment, and Booker at the Telecommunications Research Establishment of the Ministry of Aircraft Production. After the War, Gold became a Fellow of Trinity College, Cambridge, and a demonstrator in the Cavendish Laboratory. Later he joined the Royal Observatory, Greenwich, as a chief assistant, and in 1957 he left for America, where he became professor of astronomy at Harvard. He is noted for the originality of his researches in astronomy, geophysics and the theory of hearing.

Booker took the Mathematical Tripos at Cambridge in 1933 and afterwards worked on the theory of electromagnetic waves in close collaboration with the radio section of the Cavendish Laboratory. He became a Fellow of Christ's College and a University lecturer in mathematics. During the War he led the theoretical division at the Telecommunications Research Establishment and was responsible for originating the 'duct' theory of super-refraction of radio waves. He returned to Cambridge after the War and played an important part in developing the theory of the scattering of radio waves from ionospheric irregularities. He left Cambridge for the United States in 1948, and became professor of electrical engineering at Cornell University. There he extended the theory of scattering to include the effect of irregularities in the troposphere, and he was one of the leaders in the team which turned this scattering to practical use in commercial 'forward scatter' radio links. He will retain his present position at Cornell in addition to becoming associate director of the new Center.

Both Booker and Gold are noted as mathematicians who can not only talk the language of the physicists but appear also to think in that language. They seem to have an intuitive grasp of fundamental principles which enables them to see at once what a piece of mathematics really implies. Together with the physicists, engineers and mathematicians at Cornell, all of whom will be called upon to help them from time to time, they will form a powerful team.

In one set of investigations the high-power radar equipment will be used to observe radio-echoes from the planets. In another it will be used for the observations of incoherent back-scatter from the electrons in the terrestrial ionosphere. Its use for the latter purpose will follow a suggestion by Gordon, which has already been tried out successfully by Bowles, that incoherent scattering might be used to investigate the upper side of the ionosphere. Gordon is to be a member of the Cornell team at the new Centre.

All radio- and space-physicists will look forward to valuable results from the new Center.

J. A. RATCLIFFE

RESEARCH BY McGILL UNIVERSITY IN BARBADOS

Bellairs Research Institute

THE Bellairs Research Institute of McGill University was founded in 1954 by the late Commander C. W. Bellairs, R.N., for the purpose of providing the University with research facilities in tropical biology. The first laboratory was put into operation in the seaside residence of Commander Bellairs in 1955. An increasing interest in the Caribbean area on the part of McGill soon made it apparent that the original quarters were not adequate for an expanding research programme. Plans were drawn up for a new building which was completed early in 1959.

While the present Institute quarters were designed primarily for the pursuit of studies in marine biology, the needs of workers in other fields of biology have not been overlooked in the construction of the building. The laboratory consists of three bright and airy work rooms with water tables and running fresh water and sea-water. There are a large concrete aquarium and outdoor tubs for the storage of freshly caught material. There are also an office, store-rooms, photographic room and microscope room. Outlets for bottled gas have been installed and the electric supply is 110 volts, 50 cycles. Accommodation, consisting of single and double bedrooms, lounge and kitchen, is available for a limited number of visiting investigators.

There are several small boats with outboard motors for collecting in shallow water and a 30-ft. diesel launch for work at sea. The launch is equipped with a winch and gear for oceanographic and plankton collections. Diving equipment, including aqualungs, an underwater camera and a variety of nets, dredges and other collecting gear, is available.

The Institute is situated on the western coast of Barbados, some seven miles from the capital, Bridgetown. A well-developed coral reef lies directly in front of the laboratory and extensive reefs lie along most of the western side. Around the rest of the island there is a wide variety of marine environments, including rocky shores and shallow-water flats of sand, mud or grass. Along the southern coast there are extensive shallow banks of corals and gorgonids, and many deep-water forms such as crinoids and antipatharians are found in 30–40 ft. of water. Deep

oceanic water comes to within a mile or two of shore and the island lies close to the convergence of the North and South Equatorial currents.

Tropical Research Laboratory

Operating now in its second year is the McGill University Tropical Research Laboratory, located at Waterford, Barbados. The laboratory, which is administered through the departments of Geography and Meteorology of McGill University, has undertaken, in its initial stage, investigations into climatology and agricultural meteorology in the Caribbean and plans later to expand into a meteorological station as well as a graduate centre in the tropics for geographical research.

Among its immediate activities is the measurement of potential and latent evaporation at a tropical station. These results, with the usual meteorological data, will be published at regular intervals each year for the information of interested research workers in the field of climatology. Results of these investigations will also be used locally in experiments planned to aid the Department of Agriculture in the expansion of irrigation and land-use schemes.

PEST INFESTATION RESEARCH, 1958

THE report of the Pest Infestation Research Board of the Department of Scientific and Industrial Research for 1958* reflects the increased concern with problems of food storage and handling in overseas territories and on board ships, and with the problems that may arise when grain is stored for very long periods under different conditions of temperature and of oxygen concentration. Two long-term experiments have been set going, in which the effect of low temperature ($4 \cdot 5 \pm 0 \cdot 5$ °C.) and low oxygen concentration (between 1 and 2 per cent), singly or in combination, on the keeping quality of dry wheat, will be investigated. A hard Canadian and a soft English wheat are being used. Chemical analyses, milling tests and bread-making tests were made initially and will be repeated at intervals over a period of ten or twelve years. Since it is quite inexpensive to maintain large stores of grain in these conditions the outcome of this investigation will be of considerable practical importance.

Films of insecticidal lacquers containing dieldrin and endrin have proved very effective in killing adult flour beetles (*Tribolium confusum*). It has now been shown that flour up to at least 2 in. deep becomes toxic to the beetles after being in contact with the lacquer for two weeks and retains this toxicity for many months. Until the amount of contamination that might occur in practice has been established, caution in the use of these particular insecticidal preparations is advocated. Whereas much is known about the susceptibility to insecticides of the insects of stored products, very little is known about the mites. An investigation of the flour mite (*Acarus siro*) is now being undertaken to make good this deficiency.

The use of gas-proof sheets for covering produce during fumigation has been increasing greatly in recent years. But users have been unable to obtain guidance on the selection of the material best suited to this purpose. Some have proved unsuitable because of lack of durability of the coating, others are highly permeable to methyl bromide and yet others become stiff after contact with liquid methyl bromide. A systematic study has now been carried out in co-operation with manufacturers, and the Laboratory should soon be in a position to put forward agreed specifications.

In addition to these problems of immediate practical importance the Laboratory has continued to produce a large amount of basic information on the insects and other organisms affecting stored products. This has consisted in detailed life-history studies in controlled physical environments, the systematics of insect pests, the response of insects to fumigants and other insecticides. In the Biochemistry Section work of even wider interest has been carried out. The physiological and toxicological studies on the mode of action of insecticides and the biochemical nature of acquired resistance to insecticides being carried out in that section is fundamental to applied entomology of every kind.

* Department of Scientific and Industrial Research. Pest Infestation Research: The Report of the Pest Infestation Research Board with the Report of the Director of Pest Infestation Research. Pp. v+55+8 plates. (London: H.M. Stationery Office, 1959.) 5s. net.

X-RAY MEASUREMENT OF THERMAL EXPANSION PERPENDICULAR TO THE LAYER PLANES OF ARTIFICIAL AND NATURAL GRAPHITES

By E. G. STEWARD and B. P. COOK
Research Laboratories, The General Electric Co., Ltd., Wembley

THERE are apparently conflicting data in the literature concerning X-ray diffraction measurements of (*a*) the thermal expansion of graphitic carbons and (*b*) the possible variation of the expansion coefficient with the proportion (*p*) of disordered (turbostratic) stacking of the layer planes.

For natural (Ceylon) graphite (*p* almost zero) over the range 15–800° C., the variation of the interlayer spacing *d* (A.) with temperature *t* (deg. C.) is given by Nelson and Riley[1] as $d = 3 \cdot 3525 + 90 \cdot 54 \times 10^{-6}\, t + 6 \cdot 33 \times 10^{-9}\, t^2$ (giving an average expansion coefficient (α) defined as $\frac{d_2 - d_1}{d_1 \Delta t}$, of $28 \cdot 5 \pm 0 \cdot 1°$ C.$^{-1}$) and by Matuyama[2] as $d = 3 \cdot 3535 + 82 \cdot 41 \times 10^{-6}\, t + 10 \cdot 30 \times 10^{-9}\, t^2$ ($\alpha = 27 \cdot 0 \pm c.\ 0 \cdot 1°$ C.$^{-1}$) (Fig. 1). If in either determination the experimental errors have been so under-estimated that the difference in the resulting values of the average expansion coefficient is not in fact significant, then the inclusion in the appropriate

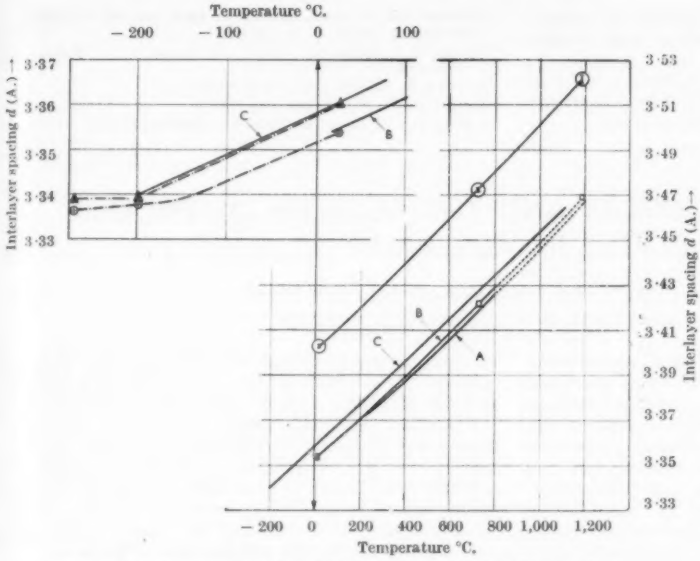

Fig. 1. Interlayer spacings of graphitic carbons as a function of temperature. *A*, $p \simeq 0$ (Matuyama); *B*, $p \simeq 0$ (Nelson and Riley); *C*, $p \simeq 0{\cdot}2$ (Walker, McKinstry and Wright); ⊕, $p \simeq 0$ (Baskin and Meyer); ▲, $p \simeq 0{\cdot}2$ (Baskin and Meyer); – – –, extrapolations to 1,200° C. from given equations; □, denotes present results and error limits for $p \simeq 0$; I, denotes present results and error limits for $p \simeq 0{\cdot}75$; – · – · –, see text

equation of the small term in t^2 would not be justifiable.

For an artificial graphite with about 20 per cent disordered stacking ($p \simeq 0{\cdot}2$) over the range −196–1,118° C., Walker, McKinstry and Wright[3] obtain $d = 3{\cdot}358 + 95{\cdot}2 \times 10^{-6}\ t$ (Fig. 1) with a standard deviation from linearity significantly smaller than the above βt^2 terms at high temperature. From their results Walker *et al.* calculate a decrease of 13 per cent in interlayer binding-energy between room temperature and 1,118° C. From a comparison of their results with those of Nelson *et al.* for the more perfect graphite, they conclude that the increase in d for a given rise in temperature is independent of p. By using the known[4] relationship between d and p one could then deduce that the expansion is independent of d—as found experimentally by Walker for their own artificial graphite.

Some recent qualitative work by Matuyama[5] on a range of essentially non-graphitic carbons is inconclusive on the issues under discussion.

Baskin and Meyer[6] report considerable differences in the amounts of crystal structure contraction of artificial ($p \simeq 0{\cdot}2$) and natural ($p \simeq 0$) graphite on cooling from room temperature to 4·2° K. (Fig. 1). Their 'average' expansion coefficient (based on two points 78° K. and 297° K.) for the artificial graphite agrees well with Walker's value but for natural graphite is lower than Nelson and Riley's value over 15–800° C. They suggest that the smaller changes they observe in cooling from 78° K. to 4·2° K. result from anisotropic contraction of crystallites producing mutually restraining stresses; these would be greater for artificial graphites having smaller crystals than for natural graphite.

To minimize dependence on absolute accuracy in making comparisons between different types of graphite, we have made measurements in the same apparatus under the same conditions, on both a natural graphite ($p \simeq 0$) and on a coke. To enhance any differences dependent on crystal structure stacking disorder, a coke with a large proportion ($p \simeq 0{\cdot}75$) of turbostratic structure was chosen. Results for room temperature, 728° C. and 1,188° C. (actual values (± 6°C.) determined by platinum lattice expansion calibration), are shown in Fig. 1. For the natural graphite where all reflexions arise from ordered structure, $000l$ and $11\bar{2}l$ reflexions were used and correction was made in the conventional way for absorption, etc. For the less graphitic material, although full correction to put the results on an accurate absolute scale was not possible, relative changes in 0004 reflexion angles were measured with sufficient precision for this initial survey. Thus, while the gradient for $p \simeq 0{\cdot}75$ has an accuracy limited only by the relative errors (indicated in Fig. 1) for the individual points, the numerical values for any temperature probably have a slightly greater error (± 0·005 A.). In the range 15–728° C. for $p \simeq 0$, $\alpha = 28{\cdot}4 \pm 0{\cdot}7° \text{ C.}^{-1}$ and for $p \simeq 0{\cdot}75$, $\alpha = 29{\cdot}2 \pm 0{\cdot}7° \text{ C.}^{-1}$.

Several significant features may be noted from the present results and survey.

The new measurements on the natural graphite closely follow the earlier findings of Nelson and Riley and give an indication at 1,188° C. of slight departure from linearity. For our less-graphitic material ($p \simeq 0{\cdot}75$) there is again approximate linearity and the expansion coefficient over the range 15–1,188° C. is the same within present limits of accuracy as that for the fully graphitic carbon. Thus in the range of interlayer spacings involved and over this temperature-range the coefficient of expansion perpendicular to the layer planes appears to be largely independent of d and p. The evidence of some curvature for the most perfect graphites supports the view that the coefficient of expansion must, however, be a function of temperature: further investigation at higher temperatures is clearly desirable.

For fully ordered graphite at 78° K. and 4·2° K., rather than deduce with Baskin and Meyer that the expansion coefficient is "distinctly lower", it is suggested that the variation of d with t is following the course shown thus –·–·– in Fig. 1; at these lower temperatures contraction is eventually limited by the repulsion forces between adjacent layer planes. Supporting this view is the observation that for the artificial graphite with $p \simeq 0{\cdot}2$ and correspondingly larger d, Walker's results show uninterrupted and essentially linear contraction from higher temperatures down to 78° K. At the lowest temperatures, where for either material the interlayer spacing is becoming critical, thermal contraction becomes smaller. Presence of imperfections in the structure also becomes important, and this may well explain the different ultimate behaviour reported by Baskin and Meyer between their materials: in particular, it would be expected that turbostratic stacking would

have a larger limiting *d*, since the random atomic arrangement between adjacent layers will hinder their close approach.

It is hoped to report and discuss these results in more detail elsewhere and when further investigations at high and low temperature have been made.

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[2] Matuyama, E., *Tanso*, **7**, 12 (1958).
[3] Walker, P. L., McKinstry, H. A., and Wright, C. C., *Indust. Eng. Chem.*, **45**, 1711 (1953).
[4] Franklin, R. E., *Acta Cryst.*, **4**, 253 (1951).
[5] Matuyama, E., *Nature*, **184**, 544 (1959).
[6] Baskin, Y., and Meyer, L., *Phys. Rev.*, **100**, 544 (1955).

PLANT THIOGLYCOSIDES AND THE PROBLEM OF ENDEMIC GOITRE IN AUSTRALIA

By H. S. BACHELARD and Prof. V. M. TRIKOJUS

Department of Biochemistry, University of Melbourne

Clements and Wishart[1] have suggested that an appreciable amount of endemic goitre in certain areas of Tasmania may be due to a goitrogen present in the milk of cows fed 'chou moellier'. Apart from other evidence, milk and extracts of skim milk from chou moellier-fed cows were found to depress the uptake of iodine-131 by the thyroid glands of experimental animals and human volunteers. Samples of milk from the goitrous area of Warwick (Queensland), where in spring and early summer the pastures are heavily contaminated with turnip weed (*Rapistrum rugosum*), were also shown to be goitrogenic[2]. By implication, other cruciferous weeds might be considered suspect in relationship to the general hypothesis of an association between plant thioglycosides and endemic goitre in certain circumstances.

Greene, Farran and Glascock[3], in comparing milk from cows fed hay, chou moellier or pastures lightly contaminated with cruciferous weeds, found the latter to have the highest goitrogenic activity. Factors complicating the comparison of the results of investigators in different parts of the world could be alterations in the content of thioglycosides in the plant due to differences in variety, soil and climatic conditions, or modifications of the rumen flora. On the other hand, Virtanen, Kreula and Kiesvaara[4] examined the milk of a cow 12 hr. after it had been fed moistened rape seeds (equivalent to 4·5 gm. (−)-5-vinyl-2-oxazolidinethione (goitrin[5])) and could detect only a small amount of a substance showing a similar spectral absorption curve to goitrin but with an absorption maximum at 255 mμ instead of 240 mμ.

In association with the Goitre Sub-Committee of the National Health and Medical Research Council, we have been engaged on a systematic investigation of the thioglycosides—and their aglycones—of chou moellier and of those cruciferous weeds which are prevalent as contaminants of pastures in goitrous areas of Tasmania and southern Queensland. We wish to report here some of the results which we have obtained in a study of *Rapistrum rugosum*.

The seeds of this plant yielded unexpectedly only traces of thioglycosides, whereas chromatographic examination of extracts of the mature fruit, including seeds, revealed the presence of one major and one minor component. From such extracts, after treatment with myrosin, we were able to separate the major aglycone in crystalline form (m.p. 45° C.; 1·2 gm. per kgm. dry weight) and to establish its identity as γ-methylsulphonyl-propyl-*iso*thiocyanate, a substance previously isolated from wallflower seeds (*Cheiranthus cheiri*) by Schneider[6] and named by him 'cheiroline'. Schneider was able to confirm the structure of cheiroline by synthesis (see also Kjaer, Marcus and Conti[7]. We are indebted to Prof. Kjaer for an authentic sample of cheiroline).

Cheiroline was also isolated from the tissues of *Rapistrum rugosum*—in the case of extracts of fresh leaves, 0·4 gm. crystalline aglycone per kgm. wet weight. The yields of pure aglycone from fruit and leaves, which must be regarded as minimum values, would indicate a potentially high daily intake by a cow grazing on heavily contaminated pastures.

The results of experiments to compare the action of cheiroline with that of known goitrogens are summarized in Table 1.

Table 1. Comparison of Effects of Cheiroline, Thiouracil and Goitrin on Iodine-131 Uptake (1 μc.) by the Thyroids of 100 gm. Male Rats

Expt. No.	Mean net counts per min.				Per cent control group		
	Control	Cheiro-line	Thio-uracil	Goitrin	Cheiro-line	Thio-uracil	Goitrin
1*	23,710	3,700	2,178	—	16	10	—
2*	14,893	4,339	1,710	—	29	11	—
3†	10,313	5,474	—	885	55	—	9
4†	13,369	5,071	—	1,170	40	—	9·5

Dosage: cheiroline, 5 mgm.; goitrin, 5 mgm.; thiouracil, 1 mgm.; each in 1 ml. saline. Control, 1 ml. saline. Four animals per group.
* Mixed strain, Wistar and *Rattus norvegicus*.
† Wistar strain.

The substances were injected subcutaneously 1 hr. prior to the intraperitoneal injection of iodine-131, the animals killed 3 hr. later and the thyroids dissected, weighed and digested overnight and then counted in a scintillation counter (compare ref. 8). On the basis of this test, cheiroline is goitrogenic, although the degree of response varied, with the differences most marked between the two strains of animals used. It was considered unlikely that variation in iodine intake was a factor, as the dietary pattern in our colony (Wistar strain) was very similar to that of the mixed strain obtained from another source in Melbourne, but that possibly the *iso*thiocyanate group had been modified *in vivo*, thereby introducing another variable. By contrast, the activities of goitrin and thiouracil, although measured in different strains, had approximately the same ratio (1 : 6) as that recorded by Astwood, Greer and Ettlinger[5]. According to Kjaer[9], *iso*thiocyanates are inactive or only weakly active as goitrogenic agents (see also Wagner-Jauregg and Koch[10]).

Since we have a prime interest in the behaviour, in the rumen, of cheiroline (and other thioglycoside aglycones), we examined its stability in this medium. Cheiroline was incubated with freshly taken rumen liquor, after filtration through butter muslin, as set out in Table 2.

Table 2. INCUBATION OF CHEIROLINE WITH RUMEN LIQUOR

	Blank	Controls		Treated	
		A	*B*	*A*	*B*
* Rumen liquor (ml.)	25	25	25	25	25
Buffer (0·1 *M* PO_4; *p*H 6·4)	10	10	10	10	10
Urea (mgm.)	—	60	60	60	60
Cheiroline (mgm.)	—	—	—	50	50
Incubation period (hr.)	—	2	5	2	5
† Final *p*H	—	6·8	6·9	6·8	6·9
‡ Ammonia (μmole/ml.)	13	33·5	39	25·5	38

* *p*H 6·4.
† Initial *p*H assumed in all cases as for blank (6·35).
‡ As volatilized from NaOH-treated samples.

Urea was added to produce levels of ammonium ion comparable to those attained in animals grazing during the flush spring growth. At the conclusion of the incubation period, the contents of the bottles were brought to 85° C., centrifuged, and the supernatants dried in the frozen state. When samples of chloroform extracts of the residues were applied to paper and run in chloroform/water and then sprayed with Grote's reagent[11] or ammoniacal silver nitrate solution, the control chromatograms were negative, whereas, on those corresponding to the flasks to which cheiroline had been added, this substance was absent, but there was a strong spot at R_F 0·12, with a minor spot which ran at the same R_F as cheiroline monothiourea (close to the origin). There was no difference between the chromatograms corresponding to the 2- and 5-hr. incubation periods. The combined chloroform extracts were passed twice through an alumina column with 80 per cent ethanol used each time to elute a slightly coloured upper band. The oily residue from the second eluate was washed several times with small amounts of ether and chloroform, and it was then obtained crystalline from methanol (yield about 12 mgm.). The substance slowly developed a permanent bluish green colour in dilute acetic acid with the addition of potassium ferrocyanide[12], as did also cheiroline monothiourea but not cheiroline itself. It was recrystallized from methanol (m.p. 117–119° C.), analysed (found: S = 30·5 per cent) and identified as *sym.* di-cheiroline thiourea ($(CH_3SO_2CH_2CH_2CH_2NH)_2C=S$), when compared with a synthetic sample, on the basis of R_F values—in chloroform/water (Fig. 1) and *n*-butanol/ethanol/water, 4 : 1 : 4—analysis (calc.: S = 30·3 per cent) and mixed melting point (which was undepressed). This compound separated rapidly in high yield when equimolecular proportions of cheiroline and γ-methylsulphonyl-propylamine hydrochloride were mixed in ethanolic solution in the presence of triethylamine. The synthetic material was recrystallized from methanol (m.p. 121–122° C.; Schneider[6] reported 125–126° C. (from water)).

Fig. 1. (1) Cheiroline; (2) crude chloroform extract; (3) synthetic di-cheiroline thiourea; (4) crystalline substance from chloroform extract; (5) cheiroline monothiourea. Cheiroline running with solvent front. Chromatogram sprayed with ammoniacal silver nitrate

A few disubstituted thioureas have been shown to be goitrogenic in the rat[13,14] and, in the case of dibenzylthiourea, in the rabbit[15] (by mouth but not when injected). Diethyl thiourea by mouth was found by Astwood[13] to be a more active goitrogen in the rat than thiourea. Ethyl *iso*thiocyanate is one of the numerous aglycones of this type recorded as occurring in Nature[16,17], very few of which possess a hydroxyl group in the appropriate position for spontaneous cyclization to derivatives of 2-oxazolidinethione related to goitrin. It may be noted that, although we have isolated crystalline goitrin from chou moellier seeds in amount equivalent to 2 gm. per kgm., this substance corresponds to but one of four major thioglycoside fractions present in the seed extracts.

In preliminary tests using the above-mentioned rat assay method, di-cheiroline thiourea proved to be only weakly goitrogenic (iodine-131 uptake 70 per cent of the control (dose 5 mgm.) compared with cheiroline 30 per cent (5 mgm.) and thiouracil 12 per cent (1 mgm.)). The results following other routes of administration may well be different, since the thiourea, when pure, is practically insoluble in ether and chloroform, sparingly so in methanol and appreciably soluble in water, properties which are largely the reverse of those shown by cheiroline. There are, moreover, several cases where the rat assay has been shown to be an unreliable index of goitrogenic potency in man, for example, of 1-methyl-2-mercaptoimidazole[18].

We are at present more interested in the questions as to whether the transformation of *iso*thiocyanates in rumen liquor is a general reaction, whether it occurs in the rumen *in vivo*, and, if so, whether the products can be absorbed and transferred to milk.

There are thus certain obvious extensions of the experiments reported here—and several of these are in progress—before a general concept of the transformation in the rumen of *iso*thiocyanates into goitrogenic disubstituted thioureas can be added to the hypothesis stated at the beginning of this article.

We are indebted to Dr. F. W. Clements for organizing the supply of suspected plants and their seeds; to Dr. M. T. McQuillan for her co-operation in the rat assays; to Dr. F. J. R. Hird for the ammonia values; and to the National Health and Medical Research Council for a personal grant (H. S. B.) and financial assistance towards the costs of the investigation.

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OCCURRENCE OF AN ORGAN-SPECIFIC ANTIGEN ASSOCIATED WITH THE MICROSOME FRACTION OF PLANT CELLS AND ITS POSSIBLE SIGNIFICANCE IN THE PROCESS OF CELLULAR DIFFERENTIATION

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THE microsome particles of cells have received considerable attention from biologists because they are widely believed to play a major part in protein synthesis. However, little has been done to determine their role in cellular differentiation, although several workers have postulated their probable importance in this fundamental process (most recently by Bonner[1]). New light has been thrown on this aspect of microsome physiology while employing the Ouchterlony serum–agar technique[2] to study the changes which occur in the antigens of the wheat coleoptile during growth and differentiation and the distribution of these antigens in the other organs of the wheat plant (*Triticum vulgare*).

The wheat coleoptile during growth undergoes a period of cell division followed by one of differentiation. Cell counts using the method of Brown and Rickless[3] established that cell division was completed when the coleoptile had been growing for 54 hr. after sowing at 25° C. (var. King II) and thereafter, until 96 hr., growth was associated with cellular differentiation which in the coleoptile is largely a process of cell elongation.

Using an adjuvant technique[4], an antiserum was raised in a rabbit against a phosphate buffered protein extract (M/50; *p*H 7·6) of three thousand coleoptiles, containing samples of both early and late phases of growth and differentiation. A phosphate-buffered agar plate was set up as shown in Fig. 1 with this antiserum added to the centre cup. Approximately 1 per cent protein extracts of coleoptiles of different ages from 18 to 96 hr. were pipetted in the outer ring of cups. The antigen mixtures and antibodies were allowed to diffuse towards each other in an incubator at 37° C. for 20 hr. Precipitin bands formed, and where they joined one another it was assumed that they corresponded to the same antigen.

From a study of the frequency and distribution of mitotic figures in histological sections, together with information obtained from the change in cell number during growth, it was estimated that at 18 hr. and 24 hr. after sowing about 85 per cent of the cells of the coleoptile were either potentially able to divide or were dividing, the remainder being mainly in the early stages of differentiation. At 30 hr. about 70 per cent were dividing or potentially able to divide, at 42 hr. 30 per cent, and at 72 hr. 0 per cent, when all were differentiating or had differentiated. In the extracts representing the high percentage of dividing or pre-dividing cells three precipitin bands predominated (Fig. 1), and as the proportion of differentiating cells increased so other bands assumed dominance. Thus it appears that as differentiation proceeds the

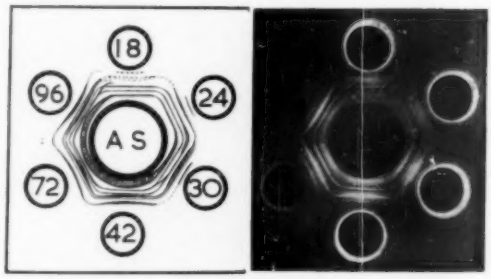

Fig. 1. Serological analyses of protein extracts representing different stages in the growth and differentiation of the wheat coleoptile. The figures in the outer ring of cups refer to the age of the coleoptile in hours from which the particular protein extract was made. The centre cup contained coleoptile antiserum possessing antibodies to all stages

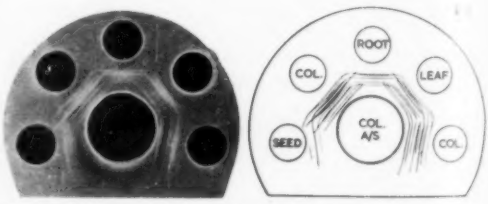

Fig. 2. Serological reactions of protein extracts made from certain plant organs of wheat seedlings, grown for 72 hr., with coleoptile antiserum. (*COL*, coleoptile; *A/S*, antiserum)

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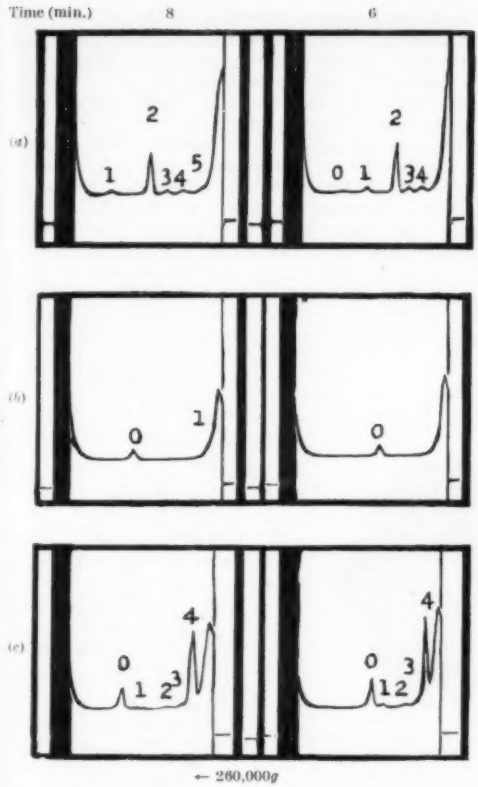

Fig. 3. Sedimentation diagrams of coleoptile, root and leaf extracts of wheat seedlings in 0·25 *M* sucrose, *M*/100 phosphate buffer *p*H 6·8. *a*, Dark-grown coleoptiles (48 hr. after sowing) ($S_{20,w} \times 10^{-13}$ values: peak 1 = 105; peak 2 = 69; peak 3 = 50; peak 4 = 33; peak 5 = 20). *b*, Roots (3 days after sowing) ($S_{20,w} \times 10^{-13}$ value: peak 0 = 73). *c*, Light-grown leaves (7 days after sowing) ($S_{20,w} \times 10^{-13}$ values: peak 0 = 74; peak 4 = 18)

principal differences in the cellular antigens are quantitative rather than qualitative. Nevertheless, it must be borne in mind that there could be qualitative antigenic differences existing between dividing and differentiating cells which were not apparent in these experiments because of the presence of a small proportion of differentiating cells in the extracts representing dividing cells.

To study the distribution of the coleoptile antigens throughout the plant, three-day-old wheat seedlings were dissected into coleoptiles, leaves, roots and seeds. These were extracted in phosphate buffer (*M*/20, *p*H 7·6) and, after centrifugation at 1,200*g* for 10 min., the extracts were pipetted into cups cut in a phosphate-buffered agar plate as shown in Fig. 2. The coleoptile antiserum was placed in the centre cup and the plate was incubated as described above. The coleoptile shared many of its precipitin lines with the leaf extract, as would be expected, since the coleoptile is in the nature of a modified leaf. The root and seed shared only a few precipitin lines with the coleoptile, although, doubtless, they possess their own specific antigens which were not detected with this antiserum.

Concurrently with these investigations an analytical ultracentrifugal study was carried out on coleoptile, leaf and root extracts. These extracts were made in phosphate-buffered (*M*/100; *p*H 6·8) 0·25 *M* sucrose and the results of the runs in the analytical ultracentrifuge are shown in Fig 3, *a*, *b* and c. All the extracts were found to contain a heavy component with a sedimentation constant within the range 69–74*S* (coleoptile = 69*S*; leaf = 74*S*; and root = 73*S*). Ts'o, Bonner and Vinograd[5] have reported that extracts of pea seedlings, after removal of the very large particles, contain a major component of 74*S*, which on analysis was found to contain 30–37 per cent ribonucleic acid, 55 per cent protein and 4·5 per cent phospholipids. They concluded that in size, chemical composition and morphology the particles resemble the nucleoproteins of the microsomes from animal tissues. Moreover, the 74*S* particle was found to dissociate at a *p*H of 8·5 into two particles of 58*S* and 38*S*, thereby forming a polymeric series[6]. Extracts were therefore made of coleoptiles (48 hr. after sowing) in 0·25 *M* sucrose at *p*H 6·8 and 8·4 (*M*/100 phosphate buffer) and it was found that the 69*S* component similarly dissociated at the higher *p*H and that the resulting $S_{20,w} \times 10^{-13}$ values of 69,50 and 33 corresponded to a polymeric series related as trimer, dimer and monomer, respectively (Fig. 4).

In order to determine whether any of the precipitin lines were associated with a 'microsome' antigen, the heavy components (mitochondria, nuclei, etc.) were first removed by a preliminary centrifugation at 10,000*g* for 30 min. from a 0·25*M* sucrose *M*/100 phosphate buffer *p*H 6·8 extract of coleoptiles grown for 48 hr. After 3 hr. dialysis against the extracting solvent, using narrow gauge dialysis tubing, the supernatant was transferred to an analytical ultracentrifuge cell and centrifuged at 260,000*g* until the 33*S* component had sedimented to the bottom of the cell. In order to clarify the serological pattern the supernatant (which should be free of the heavy components) was used to absorb out of the antiserum the antibodies specific to the relatively low molecular weight antigens, thereby producing, provided the absorption has been taken to completeness, an antiserum specific to any 'microsome' antigens. The microsome pellet was suspended in phosphate buffer

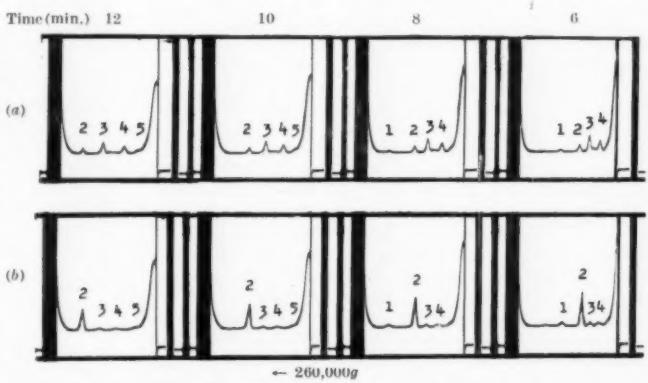

Fig. 4. Sedimentation diagrams illustrating the dissociation of the 69*S* component with *p*H change. Coleoptiles extracted in 0·25 *M* sucrose, *M*/100 phosphate buffer. *a*, *p*H 8·4; *b*, *p*H 6·8 ($S_{20,w} \times 10^{-13}$ values: peak 2 = 69; peak 3 = 50; peak 4 = 33)

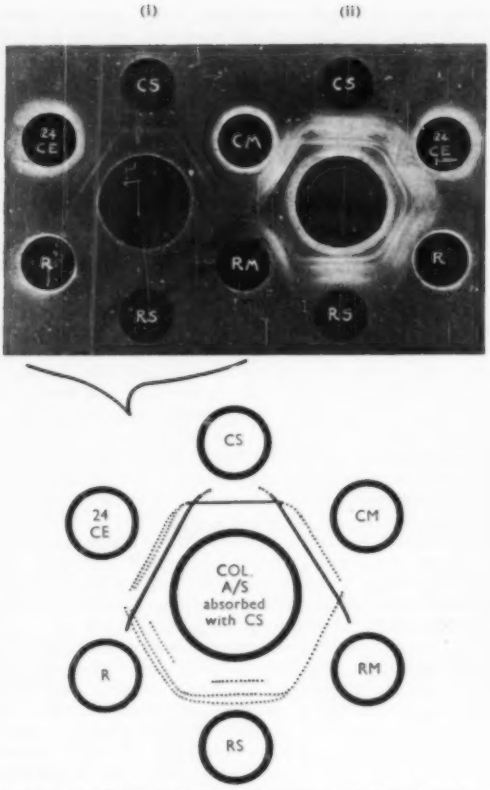

Fig. 5. Serological analyses of microsome fractions of wheat coleoptile, root and other extracts. The centre cup contained coleoptile antiserum: (i) absorbed with coleoptile extract from which the microsomes had been removed by centrifugation, and (ii) unabsorbed coleoptile antiserum. *CS*, supernatant remaining after removal of microsome particles from coleoptile protein extract by centrifugation; *CM*, coleoptile microsome fraction; *RM*, root microsome fraction; *RS*, supernatant remaining after removal of microsome particles from root protein extract; *R*, total root protein extract (that is, *RM* + *RS*); 24 *CE*, extract of coleoptiles (24 hr. after sowing) representing meristematic phase of growth

M/20, *p*H 7·6—the higher *p*H was chosen to facilitate dissociation—and the extract stored at 2° C. until required for testing.

This antiserum was afterwards tested against the microsome suspension and also a similar preparation of root microsomes. These reactions are shown with others in Fig. 5, the absorbed antiserum series on the left and the unabsorbed antiserum series on the right. To ensure that any precipitin line found to be associated with the microsome fraction was not due to incomplete absorption, a cup containing the supernatant used for absorption (*CS*) was included. The results revealed that the coleoptile microsome extract (Fig. 5i) contained two antigens when tested against the absorbed antiserum. One of these was present in the coleoptile supernatant (*CS*) and was therefore due to incomplete absorption, while the other antigen originated from the microsome fraction. In later experiments complete absorption of the antiserum was accomplished by absorbing with a larger volume of supernatant (*CS*) (Fig. 6). The position of the precipitin line associated with the microsome fraction relative to the antigen and antibody cups suggested that it was a molecule with a molecular weight of the order of thousands rather than millions. Even the monomer form of the microsome particle has a sedimentation constant in the region of 33*S*, which would imply a larger molecular weight. It would therefore appear that the 33*S* monomer must have broken down into even smaller units.

The question arises as to whether the antigenic activity resides in the typical microsome particle (69*S* + 50*S* + 33*S*) or in one or both of the 'heavy' microsome particles (peaks 0 and 1, Fig. 3*a*), or in the slower component (peak 5, Fig 3*a*). Two lines of evidence favoured the 69*S* (peak 2, Fig. 3*a*) component and its two dissociation components 50*S* (peak 3, Fig. 3*a*) and 33*S* (peak 4, Fig 3*a*) as the seat of antigenic activity; these were: (1) differential centrifugation followed by quantitative estimation[7] of the

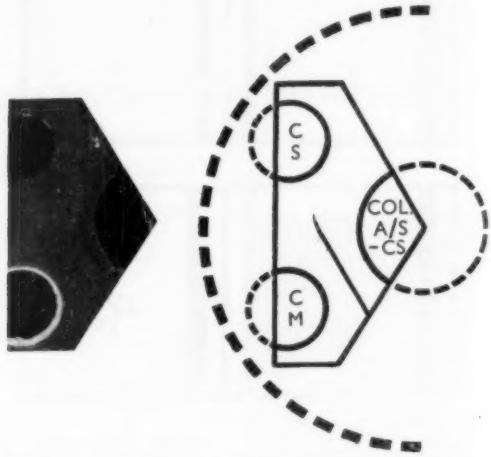

Fig. 6. Serological demonstration of an antigen associated with the microsome fraction of coleoptile protein extract. *CM*, coleoptile microsome fraction; *CS*, supernatant remaining after removal of microsome particles from coleoptile protein extract by centrifugation; *COL A/S − CS*, coleoptile antiserum absorbed with *CS*

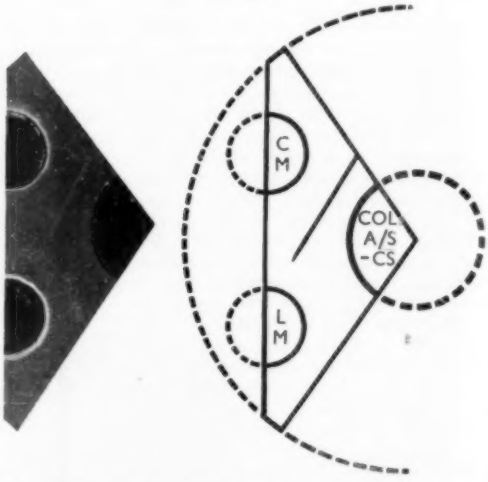

Fig. 7. Serological analyses of microsome fractions of wheat coleoptile and leaf with specific microsome fraction antiserum (that is, coleoptile antiserum (*COL. A/S*) absorbed with the supernatant (*CS*) remaining after the microsome particles have been centrifuged from a coleoptile protein extract). *CM*, coleoptile microsome fraction; *LM*, leaf microsome fraction

distribution of the antigen component; (2) the fact that the antigen still remained in the supernatant under conditions when particles of 100*S* and above (that is, including peaks 0 and 1, Fig. 3*a*) would have centrifuged down. It is quite possible that peaks 0 and 1 are higher polymers of the 69*S* particle, but in these experiments the centrifuge speed was too high to determine the $S_{20,w}$ values accurately for these two peaks. Petermann *et al.*[8] have suggested a similar hypothesis for the 'heavy' microsomes of liver extracts.

The precipitin band associated with the microsome fraction of the coleoptile extract was absent from microsome fractions of root (Fig. 5i) and leaf (Fig. 7). With the coleoptile antiserum used in these experiments any organ-specific antigens associated with root and leaf microsomes would not be detected; but it seems probable that such antigens exist.

A further point of interest was the fact that an extract made from coleoptile cells during their meristematic phase of growth (24 *CE*, Fig. 5, ii) shared nearly all its precipitin lines with the root extract (*R*, Fig. 5, ii). The exceptions were those associated with the high molecular weight components. Thus it appears, with these exceptions, that a non-organ specific meristematic pattern of antigens has superimposed upon it, during differentiation, a combination of proteins characteristic of differentiated cells.

In conclusion, it is possible to visualize the primordial cells of an organ as containing their own characteristic microsome particles as well as many antigens possessed in common with other organs. Possibly also, organ-specific microsomes synthesize organ-specific proteins or a particular combination of non-organ-specific proteins, which would either, by their incorporation into the primordial cytoplasm, or by their enzymatic activity, lead to greater cellular specialization and thereby forward the process of cellular differentiation.

I wish to express thanks to Dr. P. G. H. Gell for his many valuable suggestions throughout the course of this work, to Dr. D. R. Stanworth and Miss P. Ratcliff for carrying out the analytical ultracentrifuge determinations and to Miss A. Hudson for technical assistance demanding much patience.

These results will be reported in detail elsewhere. The work was carried out under a grant from the Nuffield Foundation.

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SPECTROPHOTOMETRIC MEASUREMENT OF CYTOCHROMES IN THE RED AND THE WHITE MUSCLES OF THE AMERICAN COCKROACH, *PERIPLANETA AMERICANA* L.

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SINCE the early report by Sacktor and Bodenstein[1], the red muscle of the male and the white one of the female American cockroach, *Periplaneta americana* L., have long been a subject of investigation, and parallelism between the coloration and the activity of oxidative enzymes of the muscles has been reported[2]. However, ordinary spectrophotometric measurement of the coloration was precluded by the turbidity of muscle. In the present experiment, the opal glass transmission method of Shibata[3] was adopted, instead of the usual spectroscopic method, which is subject to rather large errors, in an attempt to obtain quantitative or at least semi-quantitative data on the suspected difference in the content of cytochromes between the red and the white muscles of the American cockroach.

For observation of the muscle, red thoracic trochantinal and coxal muscles were collected from meso- and meta-thoraces of male adult insects; only the 'pink' muscles[4], which are identical with the red muscles of the male, were collected from the female thoraces. The muscles were soaked in 0·01 *M* diethyldithiocarbamate solution to prevent formation of melanin, which might occur. 30 mgm. of the solution was used for 100 mgm. of fresh muscle. The sample was kept in a Petri dish with a sheet of wet filter paper to avoid drying during the dissection. Then the sample was lightly ground with a small glass rod and a small amount of hydrosulphite was added.

For observation of the mitochrondria, muscles collected as above were homogenized in a glass homogenizer as a 2 per cent suspension in a solution of *p*H 7·4, containing 0·25 *M* sucrose, 0·05 *M* phosphate buffer and 0·01 *M* sodium ethylenediaminetetraacetate. On centrifuging the homogenate at 600*g* for 5 min., the precipitate was homogenized in a smaller quantity of the solution and the process was repeated five times. At the fifth extraction very few mitochondria were obtained. The resultant supernatant solution was centrifuged at 10,000*g* for 10 min. and the precipitate was washed twice by re-suspending and re-centrifuging. The procedures described above were carried out at about 4° C. The final suspension was shown microscopically to contain almost pure mitochondria, which were stained with janus green *B*. Mitochondria thus obtained show oxidative activity with α-glycerophosphate, succinate, L-glutamate, fumarate, L-malate, α-keto-glutarate and oxalacetate as substrates (un-

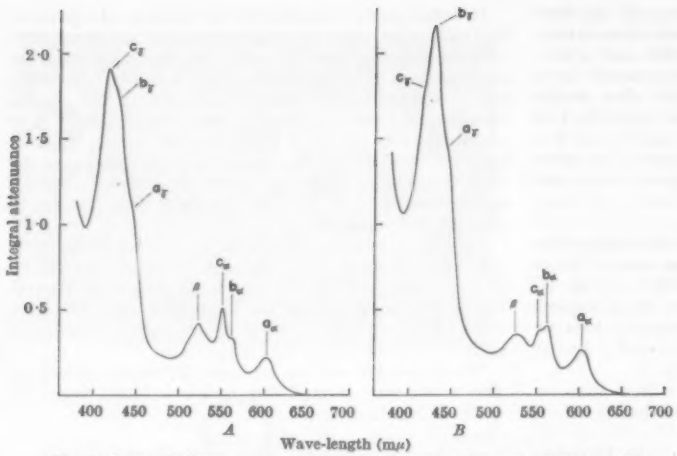

Fig. 1. Integral attenuance spectrum of (*A*) reduced red muscle of male adult American cockroach and (*B*) reduced mitochondria extracted from red muscle of male adult American cockroach, showing absorption maxima of α(a_α, b_α, c_α), β(β) and Soret (a_γ, b_γ, c_γ) bands of cytochromes

published results). The final precipitate was left to drain for 1 min. with the centrifuging tube upside down. A few crystals of sodium hydrosulphite were added to the precipitate and thoroughly mixed with it.

Although muscle and mitochondria diffused light considerably to give clear absorption spectra, opal glass was used to make sure. A brass plate, 0·6 mm. thick, with a hole a little larger than the section of the incident beam of the spectrophotometer, was placed on a plate of opal glass. The sample was put in the hole of the brass plate and a plate of transparent glass was pressed on to the sample to form an optical cell containing the sample of a definite thickness. This was mounted on an opal glass attachment and the spectrum was taken in the region 380–700 mμ. From 380 to 500 mμ black tape was applied on the reference side to lower the base-line height of the spectrum.

Since spectra of semi-integral attenuance thus obtained were lowest and constant beyond 660 mμ, this constant base-line height was subtracted to obtain integral attenuance[3], assuming that the ratio of reflectance to transmittance was approximately constant in the wave-length range observed. The spectra showed the presence of cytochromes *a*, *b* and *c* both in the muscle and in the mitochondria. Typical

Table 1. Integral Attenuance of Reduced Muscle and Mitochondria at Absorption Maxima of the α-Bands of Cytochromes

	Absorption maximum (mμ)	Integral attenuance		Male / Female
		Male	Female	
Muscle				
Cytochrome *a*	603	0·278	0·065	4·28
Cytochrome *b*	562	0·422	0·122	3·47
Cytochrome *c*	551	0·587	0·164	3·58
Mitochondria				
Cytochrome *a*	603	0·259	0·181	1·43
Cytochrome *b*	562	0·399	0·305	1·31
Cytochrome *c**	551	0·357	0·297	1·20
Dry matter of mitochondria (per cent)		19·5	18·6	

* Values for cytochrome *c* were reduced by washings.

spectra of the muscle and the mitochondria of the male cockroach are shown in Fig. 1. Spectra of the female preparations were qualitatively much the same as those of the male.

Though the absorption maximum of cytochrome *b* at about 562 mμ was obscured by the distinct absorption band of cytochrome *c*, which has its maximum at about 551 mμ, the attenuance at 562 mμ is considered to represent roughly the amount of cytochrome *b*, since cytochrome *c* absorbs light very little at this wave-length. For example, absorption at 562 mμ is about 10 per cent of that at 550 mμ in the case of the cytochrome *c* extracted from the thoracic muscle of the horn beetle, *Xylotrupes dichotomus* L. (unpublished results). In the case of mitochondria, however, cytochrome *c* was considerably washed out and the peaks of cytochrome *b* became rather clear both in the α region and in the Soret region; and cytochrome *b* was seen to remain in the mitochondria as well as cytochrome *a*. Experimental results are given in Table 1.

In all cases with muscle, the attenuance ratio of male to female was higher for cytochrome *a* than for cytochrome *c* or *b*. Though the values vary somewhat, probably with age of cockroaches, it is apparent that red muscle contains a much greater quantity of cytochromes than white muscle. On the other hand, there was no great difference in the concentration of cytochromes in mitochondria between male and female, both on a wet- and a dry-weight basis. As for the nature of mitochondria, it is not yet determined whether the mitochondrial fraction sedimented by the procedure employed is the only fraction that contains cytochromes, especially *a* and *b*. However, it seems likely that the difference in the content of cytochromes between the red and the white muscles is largely due to a greater amount of mitochondria in red muscle than in white muscle, and not so much to the difference in the concentration of cytochromes in mitochondria. In fact, red muscle yielded more than three times as much mitochondria as white muscle, both on a wet- and a dry-weight basis.

Though further experiment must be carried out to determine what substances contribute to the reddish coloration of the muscle of the American cockroach, I believe that cytochromes *a*, *b* and *c* are largely responsible. So far, I have not been able to detect any other substance in the intact and reduced muscles from their spectra.

Experiments are now in progress by this method to compare the content of cytochromes in muscles of various species of insects, and the results will be published elsewhere.

I thank Dr. J. Fukami, of this Laboratory, Mr. S. Matsunaka of the Section of Physiology and Genetics, for advice, and Dr. H. Ishikura, chief of this Laboratory, for his interest and encouragement.

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LETTERS TO THE EDITORS

COSMOLOGY

Pasteur and Cosmic Asymmetry

Yang and Lee's[1] discovery of the non-conservation of parity in certain interactions of "fundamental particles" has led to a general acceptance of the notion that the structure of the universe is asymmetrical. So far as I know this was first enunciated by Pasteur[2]. The following are his exact words:

"Il faut de toute nécessité que des actions dissymétriques président pendant la vie à l'élaboration des vrais principes immédiats naturels dissymétriques.

"Quelle peut être la nature de ces actions dissymétriques? Je pense, quant à moi, qu'elles sont d'ordre cosmique. L'univers est un ensemble dissymétrique et je suis persuadé que la vie, telle qu'elle se manifeste à nous, est fonction de la dissymétrie de l'univers ou des conséquences qu'elle entraîne. *L'univers est dissymétrique.*"

The first sentence is, I think, the first enunciation of the principle that an asymmetrical phenomenon must have an asymmetrical cause. It would be of great interest to know why Pasteur, who believed that there was a sharp gap between vital and non-living processes, attributed the asymmetry to the universe, and not to a 'vital force' or some such agency acting in parts of it. Pasteur's conjecture may of course be disproved in either or both of two ways, namely by the discovery on other planets of living beings composed mainly of sugars and amino-acids of the D series, or by the synthesis of such beings by men. But until such disproof is forthcoming he deserves full credit for his formulation.

I have to thank Prof. Jacques Monod, of the Institut Pasteur, for most kindly furnishing me with Pasteur's exact wording, and for a valuable comment.

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Nov. 18.

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GEOPHYSICS

Effect of Latitude on the Diurnal Maximum of 'Dawn Chorus'

IT is known that chorus, or 'dawn chorus', exhibits a pronounced diurnal variation in its occurrence and that different stations show different local times for the diurnal maxima. These maxima have been shown to be a function of geomagnetic latitude by Allcock[1] and independently by Pope[2].

The observations used were samples taken at 3-hr. intervals, with a consequent limitation in time resolution (excepting those obtained by the College station). The availability of observations from a large number of stations with a time resolution of 1 hr. during the International Geophysical Year makes it desirable to reconsider the latitude effect.

The data were obtained in various ways. The Geophysical Institute operated field-stations at College and Kotzebue. The data for Dunedin, Wellington, Stanford, Boulder, Seattle, Anchorage and part of those from Unalaska were supplied by IGY Whistlers-West. The data from Bermuda, Washington, D.C., Norwich, Mont Joli, Knob Lake and part of those for Unalaska were supplied by IGY Whistlers-East. The Cambridge point was obtained from Storey's doctoral dissertation[3]. The time of maximum at Saskatoon was supplied by G. M. Allcock, of New Zealand. The Macquarie result was communicated directly by radio from that station.

With the exception of the last three stations, which used the occurrence of chorus, the diurnal curves were determined from hourly chorus indices. These indices are derived through a subjective estimate of the strength of the phenomenon. Using the strengths can be considered a weighting of the occurrences in favour of those close to the station. The difference between these two techniques of obtaining the diurnal maximum is probably small.

Table 1 gives the various 'whistler' stations used in this work along with the parameters involved.

Fig. 1 is a plot relating the local time of the diurnal maximum to geomagnetic latitude. The arrows attached to the data points indicate the extent and direction of correction for magnetic time. Observations from the eastern stations are plotted with solid points while those from the western stations are plotted as open points. It is apparent that the eastern stations tend to the left of the plot whereas the western tend to the right side. Thus there appears to be an 'east–west effect' in the time of diurnal maximum, which is probably a spurious effect.

Maeda[4] has considered the effect of a distortion of the Earth's magnetic field by rotation to explain the westward shift of the cosmic-ray equator noted by Simpson[5]. This westward shift would tend to reduce the east–west effect in the northern hemisphere. The Knob Lake station, being closer to the magnetic equator, would have a lower latitude, while the

Table 1

Abbreviation	Station	Geomagnetic latitude	Eccentric geomagnetic latitude	Local time maximum	No. of months
CO	College, Alaska	64·9	67·5	14·0	36
KO	Kotzebue, Alaska	63·9	67·1	13·2	13
KL	Knob Lake, Canada	66·1	68·2	8·5	4
UN	Unalaska, Alaska	51·1	54·2	6·4	9
SE	Seattle, Washington	53·8	54·5	6·3	10
ST	Stanford, California	43·8	45·7	3·9	7
BO	Boulder, Colorado	48·9	48·3	5·5	8
NR	Norwich, Vermont	55·1	52·5	6·0	14
MJ	Mont Joli, Canada	60·0	57·1	7·5	9
WA	Washington, D.C.	50·2	47·8	3·4	10
BE	Bermuda	43·7	40·7	2·7	7
CA	Cambridge, England	54·7	52·1	6·0	3
SA	Saskatoon, Canada	60·5	60·3	9·6	1
MQ	Macquarie Island	60·2	64·4	11·0	—
DU	Dunedin, N.Z.	50·5	54·3	4·9	3
WE	Wellington, N.Z.	45·2	48·9	4·2	5

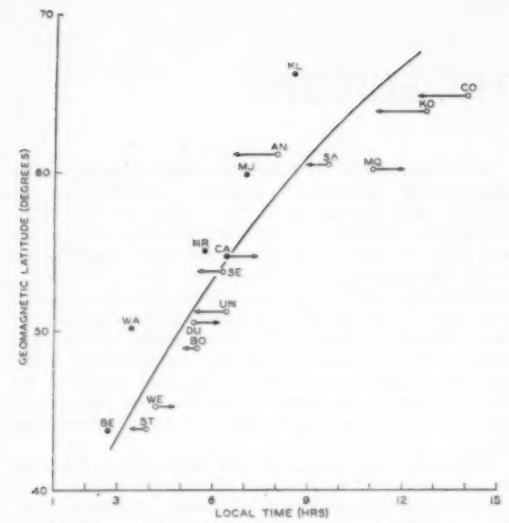

Fig. 1. Time of diurnal maximum versus geomagnetic latitude

College station, being farther from the equator, would have a higher latitude. This qualitative explanation, however, is not successful if the three southern hemisphere stations are considered, since they are all increased in latitude. Therefore, accepting the validity of the observations from the southern hemisphere stations, this westward shift of the geomagnetic equator fails to resolve the discrepancy without introducing others.

The use of geomagnetic latitudes derived from dip angles also fails to remove the discrepancy.

More success can be had by use of an eccentric dipole field. Parkinson and Cleary[6] have computed the position of the eccentric dipole-field poles for the epoch 1955. They are 81·0° N., 84·7° W. and 75·0° S., 120·4° E. Since these poles are not antipodal, the problem of computing effective geomagnetic latitudes is complicated. A simple approximation can be obtained, however, by computing the latitudes in each hemisphere separately as if the dipole were centred, using the appropriate pole. This approximation will, of course, produce a discontinuity at the equator. The latitudes using this approximation are tabulated and the results plotted in Fig. 2. The magnetic time corrections are plotted in the same manner as in Fig. 1. It is clear that while some scatter remains, the east–west effect has disappeared. In particular, the Knob Lake point is brought into reasonable agreement with the rest of the observations.

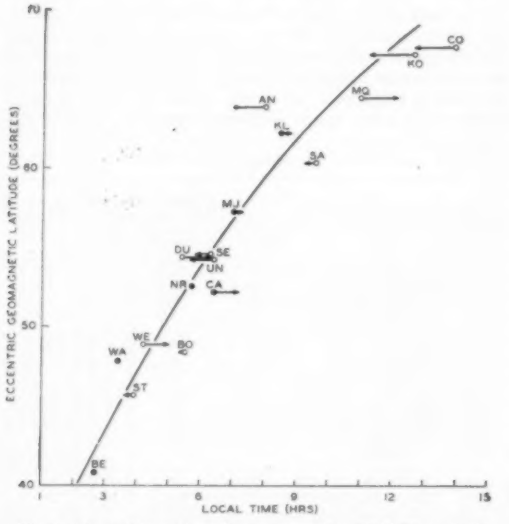

Fig. 2. Time of diurnal maximum versus 'eccentric' geomagnetic latitude

These results indicate that it is desirable to consider the use of the eccentric dipole field in connexion with theories of chorus. Also, the paths of whistler propagation may be affected by the eccentric field rather than the centred field, which may be an important consideration from the point of view of conjugate point experiments and electron density computations.

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Calculation of Cosmic-ray Ages in the Iron Meteorites 'Carbo' and 'Treysa'

In a recent communication[1], Dr. R. R. Marshall estimates the cosmic-ray age of the iron meteorite 'Carbo' to be $0{\cdot}69 \times 10^9$ yr. His calculation was based upon the following experimental results of ours[2] and Barr's[3]. We showed that the relative isotopic abundances of potassium from a sample of the iron meteorite 'Carbo' were markedly different from those of terrestrial potassium. Considerable enrichment of the isotopes of masses 40 and 41 was found ($^{39}K_m = 79{\cdot}5$ per cent, $^{40}K_m = 4{\cdot}8$ per cent, and $^{41}K_m = 15{\cdot}7$ per cent as compared to 93·08, 0·012 and 6·91 per cent respectively for ordinary terrestrial potassium). Evidence was given that a considerable amount of this meteoritic potassium—nearly all the potassium-40 and more than half the potassium-41—is of cosmogenic origin, that is, it is a reaction product of the spallation of mainly iron nuclides, induced by high-energy particles from cosmic radiation. Barr[3] measured the cross-sections for the production of fifty-nine radioactive isotopes formed by 5·7-BeV. proton bombardment of copper. From his summary of measured and interpolated independent cross-sections, Marshall calculated the total cross-sections for the production of the potassium isotopes to be $\sigma_{39} = 14{\cdot}8$ mb., $\sigma_{40} = 9{\cdot}4$ mb., and $\sigma_{41} = 14{\cdot}7$ mb.

The method of determining the cosmic-radiation age of meteorites is based on the fact that a stable and a radioactive isotope (potassium-41 and potassium-40) are formed at the same time. During the time

the meteorite is exposed to cosmic radiation, the ratio of potassium-41 to potassium-40 increases with time due to the radioactive decay of potassium-40. Besides the difficulty of measurement of isotopic abundance on the small amounts of meteoritic potassium available, the exact determination of such a potassium-41/potassium-40 age for a given meteorite is mainly a question of knowing the ratio σ_{41}/σ_{40} of the production cross-section for potassium by cosmic-ray bombardment of iron meteorites. The use of observations from a 5·7-BeV. proton bombardment of copper for the age determination of iron meteorites can only be considered as an approximate solution of the problem.

In a re-calculation of the cosmic-radiation age from the preceding values for the relative isotopic abundances and the production cross-sections, and with a value of λ taken as $0{\cdot}54 \times 10^{-9}$ yr.$^{-1}$, we found the cosmic-ray age of 'Carbo' to be $T = 1{\cdot}36 \times 10^9$ yr., instead of $0{\cdot}69 \times 10^9$ yr. as calculated by Marshall. We both used the same formula and practically the same numerical constants, with the exception that we defined R as the ratio potassium-39/potassium-41 in ordinary potassium instead of potassium-39/potassium-40 as is defined in Marshall's paper. As this difference alone cannot explain the discrepancy in the two results of age calculation, there appears to be another error in Marshall's work which we are not quite able to understand.

As the results of our isotopic abundance measurements on the extremely small potassium samples available for measurements still show rather large statistical fluctuations[4], the uncertainties in the cosmic-radiation ages are relatively large. We calculate the radiation age T from the equation (see equation (3) in our paper[4]):

$$\frac{\lambda T}{1-\exp.(-\lambda T)} = \frac{(^{41}\mathrm{K}_m - a\,^{39}\mathrm{K}_m)/^{40}\mathrm{K}_m}{\frac{\sigma_{41}}{\sigma_{40}}\left(1 - a\,\frac{\sigma_{39}}{\sigma_{41}}\right)} \qquad (1)$$

where a is $^{41}\mathrm{K}/^{39}\mathrm{K}$ in ordinary potassium (this corresponds to Marshall's equation if $R = 1/a = {}^{39}\mathrm{K}/^{41}\mathrm{K}$ and not $^{39}\mathrm{K}/^{40}\mathrm{K}$). From the scattering in the experimental results for $(^{41}\mathrm{K}_m - a\,^{39}\mathrm{K}_m)/^{40}\mathrm{K}_m$ (1·90 ... 2·05 for potassium from 'Carbo' and 1·65 ... 1·72 for potassium from 'Treysa'), we obtain the following limits for the radiation ages of the iron meteorites 'Carbo' and 'Treysa', using the cross-sections as given above:

$$\begin{aligned} &\text{'Carbo'}: \quad T_C = (1{\cdot}05 \ldots 1{\cdot}40) \times 10^9 \text{ yr.} \\ &\text{'Treysa'}: \quad T_T = (0{\cdot}50 \ldots 0{\cdot}67) \times 10^9 \text{ yr.} \end{aligned} \qquad (2)$$

The uncertainty in the assumptions upon which the age determination is based and particularly the adoption of a relatively uncertain value for the ratio of σ_{41}/σ_{40} still require a very reserved interpretation of these ages. This is the reason why, in our own treatment of the potassium-41/potassium-40 age problem[4], we eliminated the production cross-sections. In that way we obtained the following relation for the difference of the cosmic-ray age of 'Carbo' and 'Treysa' only:

$$0{\cdot}35 \times 10^9 \text{ yr.} < T_C - T_T < 1{\cdot}25 \times 10^9 \text{ yr.} \qquad (3)$$

The rather wide range for this difference depends upon the scattering of experimental results and on the possibility that the absolute radiation age of 'Carbo' may have any value between several times 10^9 and $4{\cdot}5 \times 10^9$ yr. so long as no special assumptions are made regarding the production cross-sections. The value of the upper limit of the difference holds if the radiation age of 'Carbo' should be as high as $4{\cdot}5 \times 10^9$ yr., as is theoretically possible (although very unlikely). The estimate (3) is consistent with the preceding results (2) on the radiation ages themselves, but has a much higher degree of certainty since the statement is completely independent of the ratio σ_{41}/σ_{40}. The existence of such a difference of several hundred millions of years for the radiation ages of iron meteorites is in itself a remarkable fact.

We wish to thank Dr. F. Begemann of this Institute for valuable discussions.

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In the last sentence of the second paragraph of my original, $^{40}\mathrm{K}$ was misread for $^{41}\mathrm{K}$; the definition should read: R is the ratio of $^{39}\mathrm{K}/^{41}\mathrm{K}$. Similarly, at the end of par. 4, σ_{39} should read σ_{39}, and in par. 5, line 17, σ_{40} should read σ_{39}. An error in the numerical evaluation of the radiation age led to a figure exactly half that of the correct value. Therefore, in both par. 5, line 2, and in par. 7, line 6, 0·6, should read $1{\cdot}3_8 \times 10^9$ years. Fortunately, these corrections do not affect either the principle of the calculation or, in general, the comparisons drawn in this letter.

I am grateful to Dr. H. Voshage and Dr. H. Hintenberger for directing attention to these discrepancies.

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RADIOPHYSICS

Solar Radio Bursts and Low-energy Cosmic-rays

The recent discovery, by equipment carried in satellites and balloons, that the terrestrial atmosphere is frequently bombarded by solar protons of energies 30–300 MeV.[1,2], has given rise to much speculation concerning the origin of these low-energy cosmic rays, and their relation to solar radio bursts, flares, and terrestrial disturbances. In this communication we seek to elucidate these problems from a consideration of radio spectral observations of the Sun. The radio data were obtained at the Harvard Radio Astronomy Station, Fort Davis, Texas, with sweep-frequency receivers[3] covering the range 25–580 Mc./s. The equipment scans the entire frequency-range three times per second, and a continuous record of solar radio bursts has been obtained from sunrise to sunset for the past three years.

The observations show that the radio bursts which are associated with the ejection of low-energy cosmic rays from the Sun take the form of an intense continuum, sometimes known as spectral Type IV. This radiation characteristically covers several octaves of the radio spectrum, shows only gradual changes in intensity and frequency-range, and often lasts for many hours. It probably originates in a synchrotron mechanism[4], in which electrons with relativistic velocities are trapped in orbital motion in a magnetic

field. About 15 such bursts have been recorded at Fort Davis each year during the solar maximum.

The continuum radiation is related to the ejection of low-energy cosmic rays from the Sun by a sequence of events which commences with a solar flare, usually of class 3 or 3+ (ref. 5). The flare is followed, with an average delay of 12 min., by an outburst of continuum radiation, and approximately 1 hr. later an enhanced flux of protons arrives at the Earth. Their energies, as measured by Geiger and scintillation counters carried in balloons and satellites, lie in the range 30–300 MeV., which corresponds to particle velocities of 0·2–0·7 c. Because of their low rigidity, the protons can penetrate the terrestrial atmosphere only in the vicinity of the magnetic poles, where they give rise to ionospheric absorption of very-high-frequency radio waves over the polar cap[6,7]. These polar blackouts, resulting from enhanced ionization in the *D* region of the ionosphere, at present constitute the best parameter for measuring the arrival time of the solar particles at the Earth. The minimum time delay between the onset of the solar continuum radiation and the polar blackouts is 0·7 hr.

It may be suggested that at the time the optical flare is observed, a stream of protons and electrons is ejected violently outwards from the flare region, at a velocity of approximately 0·5 c. The electrons in this stream are afterwards trapped in orbital motion in a magnetic field in the coronal levels above the active area, and generate energy in the radio band of electromagnetic spectrum. (The strength of the magnetic field, as indicated by the splitting of slow-drift radio bursts, is of the order 1–10 gauss.) The protons are not captured by the magnetic field and continue to travel outwards at high velocity, spiralling along elongated lines of force that stream from the Sun into the interplanetary plasma[8]. A travel time of 0·7 hr. over a direct path between the Sun and the Earth corresponds to a particle velocity of 0·2 c. For spiral paths the velocities will be proportionately higher. Such velocities are in accord with those deduced from direct measurements of the particle energies.

The heliographic distribution of the flares associated with continuum radiation is given in Fig. 1, and their importance distribution in Table 1. It will be seen that there is a highly significant western excess of flares associated with the ejection of low-energy cosmic rays that afterwards reached the Earth, and that most of these flares were of importance 3. The western excess has also been noted by Reid and Leinbach[7], who surmise that the connective magnetic lines of force between the Sun and the Earth emanate mainly from the western hemisphere of the Sun. These are possibly the remnants of earlier auroral corpuscular streams which have followed the well-known curved path described in the early literature by Chapman[9].

The solar protons have now been detected directly by satellite and balloon equipment on nine separate occasions. In all cases they were associated with

Table 1. Importance Classification of Flares associated with Continuum (Type IV) Radio Bursts, May 1, 1957–July 31, 1959

Importance of associated flare	Number of continuum outbursts	
	Followed by polar blackouts	Not followed by polar blackouts
3, 3+	8	3
2, 2+	2	9
1, 1+	0	7

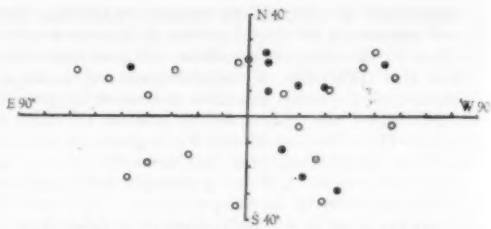

Fig. 1. Heliographic distribution of flares associated with continuum (Type IV) radio bursts, May 1, 1957–July 31, 1959. ●, Followed by ionospheric polar blackout; ○, not followed by ionospheric polar blackout. The polar blackout data are taken from Reid and Leinbach (ref. 7)

ionospheric absorption phenomena in the polar regions. The available radio data also show that the emission of these solar protons is always preceded by outbursts of continuum radiation. In approximately 75 per cent of cases they have also been succeeded, within a few days, by a geomagnetic storm, and a Forbush decrease in extra-solar cosmic-ray intensity. There is at present some disagreement concerning the relation of magnetic storms to solar radio bursts, but a detailed analysis[10] of the phenomena suggests that the emission of auroral streams, that is, particles with velocities of 1,000 km./s. (0·003 c.), is not primarily associated with solar continuum radiation, but rather with the occurrence of slow drift (Type II) radio bursts. Further analysis also suggests that noise storm bursts (Type I) and fast drift bursts (Type III) are inconsequential with regard to the terrestrial disturbances considered in this communication.

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A. Maxwell

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Nov. 14.

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PHYSICS

Variation with Temperature of the Magnetic Susceptibility of Some Transition Elements

The dependence on temperature of the magnetic susceptibilities of the transition elements has been measured over various ranges of temperatures[1], and a series of experiments is now being carried out in this Department in an attempt to confirm some of the results and extend others in the temperature range 20°–1,800° C. Preliminary results have been obtained for the elements platinum, rhodium,

Table 1

Temp. (° C.)	Susceptibility (e.m.u. gm.$^{-1}$ × 10^{-6}) Ti	Zr	Nb	Ta	Rh	Pt
20	3·35	1·32	2·28	0·85	1·05	0·97
250	3·65	1·36	2·08	0·83	1·13	0·85
500	4·18	1·39	2·00	0·80	1·25	0·73
750	4·26	1·45	1·90	0·77	1·37	0·64
1,000	5·39	1·64	1·81	0·75	1·48	0·56
1,250	5·25		1·74	0·73	1·56	0·50
1,500			1·71	0·72	1·64	0·45
1,750			1·69	0·715		0·40

zirconium, titanium, tantalum and niobium. The room temperature values of the susceptibilities (measured by comparison with platinum at room temperatures) and the values at temperature intervals up to 1,750° C. are given in Table 1.

The elements used were Johnson–Matthey 'Specpure', and no appreciable dependence of susceptibility on field-strength was observed. The error in the room temperature values is estimated as $\pm$ 0·05 × 10^{-6} e.m.u. gm.$^{-1}$. The values given in Table 1 for titanium and zirconium are in quite good agreement with the results of Squire and Kauffmann[2] (the temperatures at which the discontinuities in the χ–T curves occur being 860° C. for titanium and 880° for zirconium). The values for niobium and tantalum are similar to those of Kriessman[3].

These results supplement the evidence cited by Kriessman and Callen[1] to show that the dependence on temperature of the susceptibility of the transition elements has the same general form for each element in a particular column of the Periodic Table.

Measurements are being made on other transition elements, and a fuller account will be submitted elsewhere.

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Surface Characteristics of Fractured Poly(methyl methacrylate)

A study of fracture phenomena in poly(methyl methacrylate) has been undertaken, which involves the breaking, in tension, of strip samples, each containing a preformed crack which lies in a plane normal to the long axis (Fig. 1). The samples are extended at fixed rates of elongation until rupture ensues, invariably at the site of the initial crack. It appears by visual inspection that the fracture process occurs in two distinct stages. The crack starts to grow fairly slowly at a velocity which is of the order of 0·1 cm./sec., until a critical stage is reached when the velocity suddenly increases to a value of at least 10^4 cm./sec. It is not yet known what factor is critical in producing the transition. The final high velocity is possibly the theoretical maximum, limited by the velocity of a longitudinal wave in the medium[1], but no accurate measurements have yet been made. A sharp characteristic audible effect is associated with the rapid stage of crack growth.

On examination of the fracture surface, the regions corresponding to the above behaviour can be readily

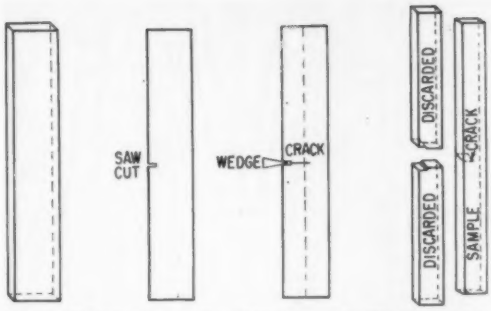

Fig. 1. Method of preparation of tensile samples (the position of the broken line in the third stage, along which the strip is cut to give the sample, determines the depth of the crack)

distinguished. Two matching fracture surfaces (that is, from the same sample) are shown in Fig. 2. In the region of slow crack growth, the surface is irregular, with deep lines of level differences, usually running in the direction of crack propagation. The increase in crack velocity is indicated by a change in the character of the surface. It becomes smoother and highly reflecting, with only shallow lines of level-differences. In this 'fast' region the degree of roughness increases, and the occurrence of the familiar parabolic figures[2] obtrudes as the distance from the initial crack increases, though this transition is gradual. The area occupied by these separate regions decreases as the length of the initial crack decreases; and in samples which do not contain deliberate cracks initially, the regions lie so close to the origin of the crack that they cannot readily be distinguished.

Perhaps the most surprising feature of the fracture surfaces formed under these conditions is the colour effects which are observed. These are readily visible to the naked eye, but are best seen under a low-power microscope (× 10) with vertical illumination. In the highly reflecting region, fringes or bands are observed, the colours of which vary in regular order with increasing distance from the initial crack. Second- and possibly higher-order 'spectra' can usually be seen before the effect is lost because of increasing roughness of the surface. Careful examination of the fringes on the fracture surface of one-half of the broken sample reveals that they do not run continuously across the surface, but occupy alternate regions bounded by the lines of level-differences.

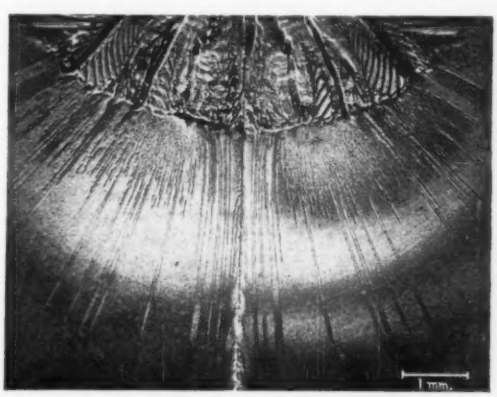

Fig. 2. Fracture surfaces of a tensile sample (green filter)

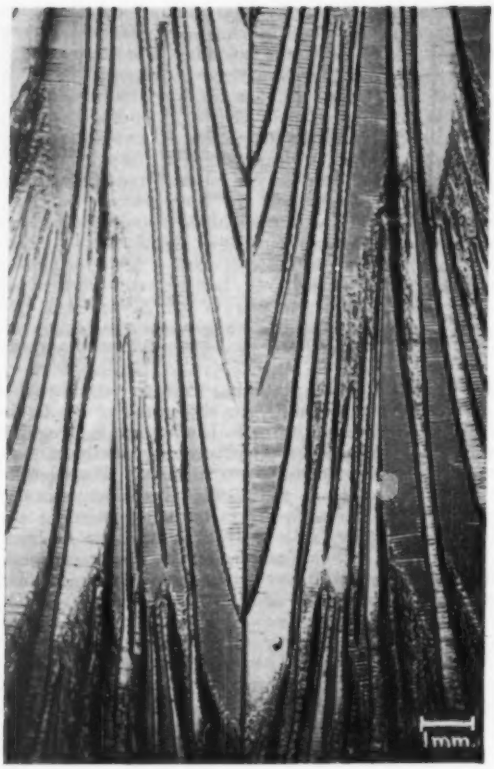

Fig. 3. Fracture surfaces of a cleavage sample (green filter)

In the intervening regions, a second set of fringes can be observed, the colours of which appear to be complementary to the first set. If the two matching fracture surfaces are examined simultaneously (Fig. 2), this duality persists in the second half of the sample, but the order of the fringes is inverted. Thus the colour fringes in the corresponding areas on the two surfaces, as defined by the lines of level-differences, exhibit the same complementarity found in adjacent regions on any one of the surfaces.

In the initial region, where the crack is growing slowly, colour effects are also observed, but they can best be produced under conditions where the rate of growth of the crack can be more readily controlled, as in a cleavage experiment (Fig. 3). In this case, fringes are not produced, and the colours are remarkably constant in hue over large areas. The same phenomenon of the complementary colours in corresponding and adjacent regions is still present, however. Colour effects have also been produced by an impact type of fracture process, as recently reported by Wolock[3].

It is believed that some of the observations noted above can be ascribed to optical interference at the surface. As the crack progresses through the (glassy) polymer, the high stresses at the tip will tend to orient the macromolecules in a direction parallel to the applied stress and hence normal to the direction of the crack and the final fracture surface. The extent to which orientation occurs will depend on the magnitude of the stress at the tip of the crack, the time for which it is applied and hence the instantaneous velocity of the crack, the temperature at the crack tip, and the viscous properties of the polymer. The refractive index along the individual polymer chains is different from that in a perpendicular direction. Hence, if the orientation of the polymer chains extends down to a specific depth into the bulk material, the result will be the same as that of a thin film on a substrate of different refractive index. This can therefore give rise to interference colours. The occurrence of colour fringes in the region of rapid crack growth can be interpreted as changes in the thickness of the film, that is, extent of orientation, as the crack progresses. In the region where the crack is growing slowly (1 mm./min. in a cleavage experiment), the film would be considered to be of remarkably constant thickness.

The orientation hypothesis is supported by other evidence. Thus the colours are thermolabile, and are destroyed by solvent in the liquid or vapour phase. There is a great discrepancy between the observed ($\sim 4 \times 10^5$ ergs/cm.^{2})[4] and calculated ($\sim 10^2$ ergs/cm.^{2}) surface energy for this material, the latter value having been computed from the energy required to rupture the chemical bonds in unit area of cross-section, assuming perfect orientation (cf. ref. 5). Clearly, a considerable amount of energy is required to produce the extent of orientation necessary to give the interference effect postulated above. However, evidence for the orientation hypothesis is still far from complete, and no reasonable explanation has been formulated for the complementarity effect which has been described above.

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Flow of Electrolyte within a Propagating Stress-Corrosion Crack

The observed corrosion potential at the mouth of a propagating crack in stressed Cu_3Au immersed in $FeCl_3$ is as much as 0·1 V. negative relative to that at uncracked parts of the surface[1]. This suggests that the advancing crack edge is dissolving anodically at an even lower potential. Potential measurements on stress-corroding wires of 18–8 steel in hot magnesium chloride solution[2,3], where the average corrosion potential during cracking is only about 0·06 V. above the reversible potential, suggest further that the potential at the advancing edges must in fact be very close to the reversible value. This led Hoar and West[4] to study the electrochemistry of 18–8 surfaces made to represent, on a macroscopic scale, the yielding material at such edges. Their experiments showed that dissolution of yielding 18–8 proceeds at the reversible potential provided that concentration over-potential is eliminated by flowing electrolyte past the surface at a suitable rate. It may be inferred that the advancing edge of a propagating stress-corrosion crack dissolves continuously into substantially virgin electrolyte, in view of the concomitant yawning.

This hypothesis has been tested by using two rather simplified crack models, and it has been found that, provided that the sides of the crack are strongly wetted, electrolyte is readily transferred from the crack mouth to the propagating edge, although in the models used some mixing with the crack electrolyte took place.

Two pieces of 'Perspex' apparatus were used: (*a*) *Apparatus A*: a curved surface is allowed to bend away from a plane one, so that the 'crack' between them progressively yawns and is propagated at 0·5–10 cm./sec. This model most closely represents a true stress-corroding crack, although the 'profile ratio' (that is, crack-length divided by the amount of yawning at the mouth) is rather smaller (20 : 1) than that normally obtaining (nearer 100 : 1 for 18–8). The crack can be propagated a total distance of 5 cm., at which point it encounters a probe Ag,AgCl electrode. Provision is made for injecting chloride ions or a suitable dye into the electrolyte just in front of the crack mouth.

(*b*) *Apparatus B*: two straight surfaces are slid over one another at a rate of 0·2–5 cm./sec. The angle of contact, which determines the profile ratio, may be adjusted to give ratios between 15 : 1 and ∞ : 1 and the probe electrode is situated always at the advancing crack edge. Again, there is provision for injecting a 'marker' solution just in front of the crack mouth. The sliding action is thought to militate against indrawing of fresh electrolyte instantaneously to the crack edge, in view of the stirring produced, so that any positive results are probably conservative.

While neither apparatus gave rise to any sudden discontinuity in the potential of the probe electrode when saturated potassium chloride flowed past the mouth of a crack being propagated in tap water as electrolyte, measurable effects occurred when 'Teepol' was present. If we convert the observed potential-changes into changes in chloride ion concentration, apparatus *A* exhibited local concentration increases at the advancing edge of between 1·1 and 1·9 times. For apparatus *B*, where the average crack length was 4–5 cm. and the propagation-rate about 2 cm./sec. (that is, some 10^4 times the propagation-rate in true stress-corrosion cracks in 18–8), the local concentration change was 1·7–4·5 times. Oddly, the concentration rise at the crack edge in apparatus *B* did not seem unduly dependent upon profile ratios up to 100 : 1, although as expected a ratio of ∞ : 1 (that is, a closed crack) led to no rise. The addition of saturated potassium chloride directly to the vicinity of a similar electrode gave a local concentration rise of about 20 times.

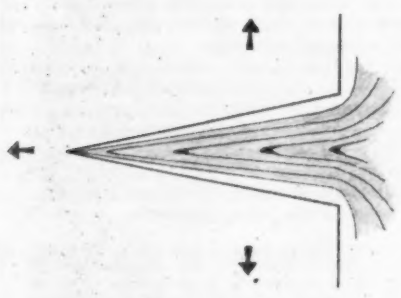

Fig. 1. Flow of electrolyte into propagating crack (with scale greatly exaggerated in the vertical direction)

With alcohol as electrolyte and deep red dye as 'marker', solution flow into a propagating crack was observed as in Fig. 1. So far as it was possible to tell, dye was conveyed instantaneously to the crack edge in apparatus *A* but results were less clear for apparatus *B*.

It may reasonably be concluded that, for long thin propagating cracks, anodic dissolution occurs into electrolyte contaminated no more than five times (the 'best' value from apparatus *B* was 20/4·5 = 4·4 times) by the previous crack contents, so long as the electrolyte thoroughly wets the sides of the crack. Owing to the inferred electrochemical nature of crack propagation in 18–8 steels[2,4], where capillarity must be such as to cause hot chloride solution to penetrate each crack right to the advancing edge, it is probable that wetting in this instance is similar to that which obtains for alcohol and aqueous solutions of 'Teepol' on surfaces of 'Perspex'.

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GEOLOGY

Radiocarbon Measurements and Annual Rings in Cave Formations

The radiocarbon method has been used to test the hypothesis that the prominent rings found in certain calcite deposits of Moaning Cave in California represent annual growth variations. Carbon-14 analyses were made on two samples taken from a presumably active section of travertine covering a human femur (Fig. 1). When discovered in 1951, the specimen had 1,206 identifiable rings with an estimated 200 additional rings obscured[1]. Thus, if these rings formed annually and without interruption the bone at the centre became the nucleus of the cave formation about 1,400 years ago.

The locations of the two samples (*L*-530*A* and *L*-530*C*) taken for radiocarbon analysis are shown in Fig. 1. The two dotted lines overlie rings which on a geometrical basis have the mean age of the analysed material. In determining the position of these dotted lines a linear rate of growth is assumed. The radiocarbon concentration of the ring corresponding to the dotted line should thus be equal to the average radiocarbon concentration of the entire sample.

In Table 1 are the results of the radiocarbon analyses, expressed as fractions of the carbon-14/carbon-12 ratio in age-corrected pre-1900 woods (tree-ring samples corrected for decay of carbon-14 between the growth date and the present). The results in column 4 are uncorrected for isotope fractionation effects, while those in column 6 are normalized to a common carbon-13 concentration based on the estimated carbon-13 values given in column 5. The results in column 6 may be thought of as those which would have been obtained if isotope fractionation did not occur in Nature.

Table 1

Sample	Location in specimen (cm.)	Depth to mean age	Fraction pre-1900 wood $^{14}C/^{12}C$	$\delta\,^{13}C$* per mil	Normalized fraction pre-1900 wood $^{14}C/^{12}C$
L-530*A*	6·1–8·8	7·5	0·931 ± 0·017	(−5)	0·890 ± 0·017
L-530*C*	0·0–2·6	1·3	0·826 ± 0·010	(−5)	0·784 ± 0·010

* Relative to the Belemnite standard.

In computing ages from these results the main problem is that the initial carbon-14/carbon-12 ratio in the samples cannot be independently estimated. As shown by Deevey *et al.*[2], freshwater carbonates can have abnormally low carbon-14/carbon-12 ratios because of the incorporation of carbon derived from old limestones. In no case, however, could the samples, when deposited, have had carbon-14/carbon-12 ratios exceeding that in age-corrected pre-1900 wood. This means that upper limits can be set on the ages of the two samples by directly inserting into the carbon-14 age equation the ratios in column 6. The results are 950 years for sample *L*-530*A* and 1,950 years for sample *L*-530*C*.

Without even knowing the original carbon-14/carbon-12 ratio of the deposited carbonate, a more meaningful estimate can be made in the following way. If in the section considered the carbon-14/carbon-12 ratio in newly deposited calcite is assumed to remain constant with time, the difference between the radiocarbon concentration in the two samples is a direct indication of the average time intervening between their deposition. The calculations indicate that the 1·3-cm. layer was deposited 1,000 ± 200 years earlier than the 7·5-cm. layer. If the rate of accumulation between the 0·0- and the 1·3-cm. levels is assumed to be the same as the average rate over the 1·3–7·5-cm. span, the time required to deposit the first 1·3 cm. of calcite was 200 years. Similar reasoning yields 200 years for the time interval between the 7·5-cm. level and the surface of the specimen (8·8 cm.). Hence, based on the assumptions of constant initial carbon-14/carbon-12 ratio and of a linear growth-rate, the accumulation period for the entire 8·8 cm. of travertine is estimated as 1,400 ± 250 years. It should be noted that this conclusion is independent of the time at which deposition ceased.

The further assumption that the formation was actively growing when the specimen was collected makes possible an estimate of the initial carbon-14/carbon-12 ratio. In this event the 7·5-cm. level would have an age of 200 years and the 1·3-cm. level an age of 1,200 years. This assumption fixes the initial carbon-14/carbon-12 ratio of newly deposited carbonate at 0·915 that in age-corrected pre-1900 wood.

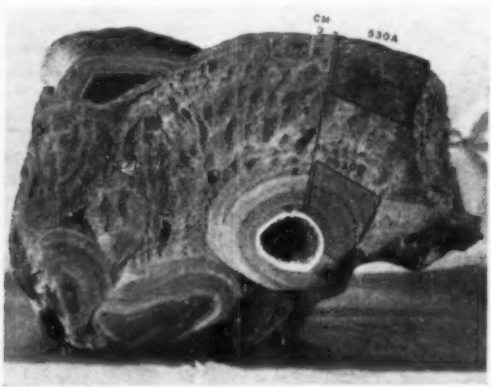

Fig. 1. Photograph of section cut through travertine deposit. The location of the samples analysed for carbon-14 are outlined. The age computed for the dotted mid-line for sample *L*-530*A* is 200 ± 100 and for the dotted mid-line of *L*-530*C* is 1,200 ± 200

It is of interest to compare this result with that obtained on a sample of recently formed calcite from Crystal Palace Cave located in Tuolume County, California. Supplied by R. de Saussure, this sample consisted of calcite covering and replacing a piece of wood found in the cave. A radiocarbon analysis of the wood yielded an age of less than 150 years and confirmed de Saussure's contention that it was a recent addition to the cave. The carbon-14/carbon-12 ratio for the calcite was 0·937 (± 0·008) that in age-corrected pre-1900 wood. When normalized for isotope fractionation, the result becomes 0·901 ± 0·008 (based on δ-carbon-13 = − 7·2‰)—in good agreement with the value of 0·915 required for the Moaning Cave samples.

In the literature, most of the published results for terrestrial waters are less than 0·90. For example, Deevey *et al.*[2] give values around 0·80 for alkaline lakes, while Brinkman, Munnich and Vogel[3] cite values of 0·66–0·84 for alkaline ground water. In addition, Broecker and Walton[4] give 0·67 as the average for the dissolved carbonate in rivers draining sedimentary areas. However, Broecker and Walton[4] find values of 0·90–0·98 for streams draining igneous areas devoid of carbonate rocks. This difference between 'igneous' and 'sedimentary' rivers arises from the fact that the bicarbonate in rivers draining only silicate rocks is almost entirely from soil carbon dioxide which has reacted with silicate minerals, whereas in carbonate areas the bicarbonate of the carbon is derived partly from soil carbon dioxide and partly from limestone free of carbon-14. (Soil carbon dioxide has a carbon-14/carbon-12 ratio approximately equal to that in contemporary wood.) Although the samples reported here are from limestone caves, the waters entering these caves apparently have derived nearly all their bicarbonate from soil carbon dioxide.

In conclusion, then, the radiocarbon results suggest that the rings observed in the travertine are annual, probably reflecting seasonal changes in the mode of deposition. This is consistent with Orr's[1] study of calcite coatings on nails which had remained in Moaning Cave for 29 years (1922–51). Using high magnification he found up to 29 rings. Whether this applies to cave formations in general awaits similar studies on other deposits. The present study merely demonstrates how the radiocarbon method can be applied to such problems.

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CHEMISTRY

Substitution Reactions of Cobaltic Complexes catalysed by Adsorption

THE rate of the reduction of the complexes $[Co(NH_3)_5X]$ at the dropping mercury electrode depends on the nature of the supporting electrolyte and increases in the order: $SO_4^= \ll ClO_4^- < NO_3^- < Cl^- < Br^-$ as is manifested by the shift of the half-wave potential of the reduction wave with the change of the supporting electrolyte. The halides Cl^-, Br^- (and under suitable conditions also I^-) cause a further, quite different effect: at a certain concentration of the halide ion a new reduction wave appears which starts from the potential of the dissolution of mercury. The relative height of this wave (that is, the ratio of the positive wave to the total diffusion current) increases non-linearly with increasing concentration of the anion until all the complex is reduced in this positive wave (Fig. 1).

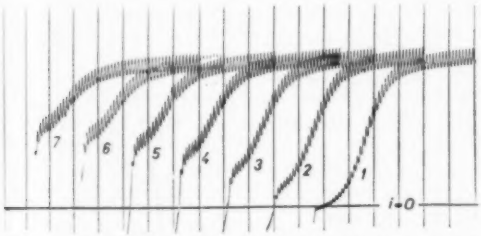

Fig. 1. The influence of Br^- ions on the reduction wave of $[Co(NH_3)_5.H_2O]^{3+}$.
Solution: 0·1 *M* $HClO_4$, 0·9 *M* $NaClO_4$, 2×10^{-3} *M* $[Co(NH_3)_5.H_2O](ClO_4)_3$.
Curve 1, 0; 2, 2×10^{-4}; 3, 4×10^{-4}; 4, 6×10^{-4}; 5, 8×10^{-4}; 6, 10×10^{-4}; 7, 12×10^{-4} *M* NaBr.
100 mV./Absc., from −0·2 V. against mercurous sulphate electrode; $S = 1:40$

The concentration of the anion necessary for the development of the positive wave of given relative height depends on the nature of the anion: Cl^- ions have a much smaller effect than Br^- ions. Also the nature and concentration of other anions present in the solution influence the relative height of the positive wave, the latter being highest in solutions of pure halides and decreasing by addition of ClO_4^-, NO_3^- or $SO_4^=$ ions.

This behaviour suggests that the addition of halides changes the mechanism of the reduction of the cobaltic complex. This is further supported by the fact that the variation of the positive wave with the height of the mercury reservoir shows that, if the relative height of the first wave is small, the transport of the depolarizer to the electrode surface is governed not by diffusion but rather resembles that governed by a reaction preceding the electrode reaction proper. Furthermore, the properties of the positive wave are almost identical with those of the wave of $[Co(NH_3)_5Cl]^{2+}$ or $[Co(NH_3)_5Br]^{2+}$ (Vlček, A. A., unpublished results), so that these ions are probably the actual depolarizing particles. From this it could be concluded that in presence of halides the reaction:

$$[Co(NH_3)_5X]^{2+} + Cl^- \underset{k}{\rightarrow} [Co(NH_3)_5Cl]^{2+} + X^- \qquad (1)$$

(or similarly with Br^- or I^-) occurs in the solution surrounding the electrode and furnishes the actual depolarizer. Then the height of the first wave would be given by the rate of the homogeneous reaction (1). The value of the rate constant k measured by other methods[1] for the reaction of the $[Co(NH_3)_5H_2O]^{3+}$ with Cl^- ions is of the order of $10^{-5}(mol./l.)^{-1}s^{-1}$, and it is thus impossible that the reaction in the solution near the electrode would contribute to the positive wave: the rate constant necessary for the observed currents would have to be at least 10^7 times higher than that measured in the solution.

To explain all the observed phenomena it has to be assumed that the particle actually exchanging the electrons with the electrode at the potential of the first wave is supplied by a chemical reaction proceeding directly at the electrode surface, the reactants being in the adsorbed state. This assumption that the reaction proceeds as a heterogeneous one is supported by the study of the current-time curves at constant potential. For small relative heights these curves obey the equation $i = \text{const.} \times t^{\alpha}$, where $\alpha > 2/3$, which is a property characteristic of processes in which absorption plays a decisive part[2]. The influence of surface-active substances, in the presence of which the relative height of the first wave is diminished, points to the same conclusion.

When a reaction proceeds directly at the electrode surface, all the particles formed react immediately with the electrode. On the other hand, the amount of particles formed per unit time in the adsorbed monomolecular layer is much smaller than that in the volume of the solution so that the assumption that the reaction proceeds in the adsorbed state would not explain the observed phenomena. Hence a further assumption has to be made, namely, that the reaction proceeds by a quite different rate, being catalysed by the adsorption at the surface of the electrode. Such catalysis of reactions of complexes has been observed in many cases (for review see ref. 3), the catalyst being charcoal, silica gel, mercury, Raney nickel, etc. The reaction which leads to the appearance of the positive wave is of a similar nature. It is a substitution reaction catalysed by the adsorption at the surface of the electrode.

The polarographic method can thus be used for the study of the ability of various complexes to undergo these heterogeneous substitutions, the relative height of the first wave being a measure of the rate constant of the reaction. For a series of complexes $[Co(NH_3)_5X]$ the following order of the reaction rates with Br^- ions in 0·1 *M* $HClO_4$, 0·9 *M* $NaClO_4$, 5×10^{-3} *M* NaBr solution has been deduced, X being:

$$NO_2^- < NH_3 \approx F^- < Ox^= \approx (ONO)^- < CH_3COO^- < H_2O < NO_3^- \qquad (2)$$

The adsorption catalysis is probably due to the strong polarization of the complex owing to the formation of a bond with the catalyst and to the influence of the electric field in the interface between the catalyst and the solution. The activation energy of a process is much decreased by such a polarization, the complex being much more reactive in the adsorbed state. This mechanism is identical with that which is supposed to operate in the electrode reactions[4], as can be seen also by the close parallelism between the rate of the catalysed substitution reactions and the rate of the electrode reactions; the order of the latter being in 1 *M* $NaHSO_4$ (ref. 4 and Vlček, A. A., unpublished results):

$$NH_3 < F^- < Ox^= < (ONO)^-, NO_2^- < CH_3COOH < H_2O < NO_3^- \qquad (3)$$

With the exception of the complex with nitrite, the two series are almost identical.

This close parallelism suggests that the structural changes of the reacting complexes, that is, the configuration of the activated complex, are very similar in both cases. For the electrode reaction it has been found[4] that, with the exception of the complex $[Co(NH_3)_5NO_2]^{2+}$ (Vlček, A. A., unpublished results), the complexes are orientated in such a way that the atomic group X is as distant as possible from the electrode surface. This orientation is also very convenient for the substitution reaction, the heterogroup being accessible for the exchange with other reactants without any bond breaking and rearrangement of the complex.

The proof of substitution reactions catalysed by adsorption at the electrode surface is very important for the interpretation of many observations concerning the influence of anions on the electrochemical behaviour of complexes. The presence of many anions causes a great shift of the reduction wave of some complexes towards positive potentials. This shift may be due to a substitution reaction of the type here described, which, when rapid, causes the reduction of the total amount of the depolarizer to take place at positive potentials.

The experimental details and further discussion will be published elsewhere.

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Microwave Spectrum of Fluorine Cyanide

A fraction of the product of fluorination of cyanogen, in which fluorine cyanide was detected by infra-red spectroscopy[1], has now been shown to absorb in the microwave regions of 21,000, 42,000 and 63,000 Mc./s. The absorptions occur in the frequency-ranges expected from the resolved rotational structure of the infra-red band, ν_2, centred on 1,077 cm.$^{-1}$. Their Stark effects and nuclear quadrupole splittings are those expected for $J = 0 \rightarrow 1$, $1 \rightarrow 2$ and $2 \rightarrow 3$ transitions in the linear molecule, $FC^{14}N$. Spectra due to molecules with one quantum of vibrational energy in the degenerate bending mode (ν_2) have also been measured.

Values so far obtained are the rotational constant, $B_0 = 10{,}554{\cdot}20 \pm 0{\cdot}01$ Mc./s.; the coupling constant for $^{14}N = -2{\cdot}67 \pm 0{\cdot}05$ Mc./s.; the dipole moment, $\mu = 1{\cdot}68 \pm 0{\cdot}05$ D; and the centrifugal stretching constant, $D_J = 0{\cdot}0053 \pm 0{\cdot}0003$ Mc./s. The quadrupole coupling suggests a C—N distance slightly more than 1·163 A. found[2] in ClCN: if 1·165 A. is assumed, the C—F distance is obtained as 1·260 A. This is the shortest C—F distance known, and implies, in agreement with the low value of the coupling constant, an important contribution of $^-F{=}C{=}N^+$ to the structure.

We are at present attempting to improve the yield and the purity of the sample. It should then be possible to detect other isotopic species in natural abundance (particularly $FC^{15}N$). We hope to be able to detect and resolve other infra-red bands to see if the small discrepancy between the microwave and the infra-red (parallel mode) B-values extends to infra-red values obtained from perpendicular modes.

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Thiol Exchange System with Thiolbenzoic Acid

Although many methods for the preparation of thiolacetic acid have been reported[1], all rely on the use of hydrogen sulphide, preferably under pressure, or phosphorous pentasulphide, which is not pleasant to handle.

It occurred to us that if an equilibrium existed between thiolbenzoic acid and thiolacetic acid:

$$C_6H_5COSH + CH_3COOH \rightleftharpoons C_6H_5COOH + CH_3COSH$$

the reaction might be persuaded to go towards completion by removal of the latter as the lowest-boiling component.

We have, in fact, obtained thiolacetic acid in yields of 95 per cent, based on the thiolbenzoic acid used, by this exchange method which we believe to be novel. Catalytic amounts of water appear to be essential; the most satisfactory method is to reflux glacial acetic acid (2 mol.) under a packed column fitted with reflux head, then gradually (8–12 hr.) add to the still, thiolbenzoic acid (1 mol.) previously saturated by shaking with water. The head temperature falls to 87–89° C. and the thiolacetic acid is withdrawn at such a rate that this temperature is maintained. The product needs drying for storage.

As thiolbenzoic acid is now commercially available the method should be attractive to those wishing to prepare conveniently relatively small quantities of thiolacetic acid.

Thiolpropionic acid was prepared by the same method, but attempts to convert butyl alcohol to butyl mercaptan were unsuccessful.

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RADIOCHEMISTRY

An Alternative Mechanism for Chemical Protection against Radiation Damage

Studies of the effects of chemicals in decreasing the radiation damage suffered by exposed organisms have revealed a number of compounds which are quite efficient in this respect[1–3]. While several mechanisms have been suggested for their mode of action[4], none has been universally accepted. It seems possible that the protective action may function

through several alternative routes. It is the purpose of this communication to show another possible mode of action of these agents and some of the evidence which supports it. The mode of protection cited here need not be an exclusive one.

Almost all the best protective agents have structures characteristic of good chelating agents. The general structures of many of these agents can be written as $X—CH_2—CH_2—Y$ or $X—CH_2—CH_2—CH_2—Y$, where X and Y are groups capable of co-ordinating with one or more of the co-ordination sites of a metal ion. Thus these are compounds which can readily form five- or six-membered rings upon chelation. Typical examples of such compounds are β-mercaptoethylamine, glutathione, 2,2′-dithioethanol, 3-mercaptopropylamine and S,2-aminoethyl*iso*thiourea. It is suggested that their effectiveness has the following basis. In the course of radiation some of the transition metal constituents of the organism are ejected from their normal sites by the radiation. These are then more or less free to enter the circulation and do so in an amount sufficiently large to poison many of the metal ion-inhibited enzymes such as adenosine triphosphatase, carbonic anhydrase, arginase, etc.[5]. In the presence of the protective agent the 'outlaw' metal ions are tied up in the form of chelates which are more stable than the complexes formed with the enzyme. Thus no inhibition of such enzymatic processes can occur. Since many such inhibition processes are irreversible, the ineffectiveness of administering the protective agent after irradiation is explicable.

If such is the case, a correlation between the stability of the resulting chelate and the effectiveness of the protection furnished would be expected. Unfortunately, quantitative studies of both stability constants and survival rates are available on only a few compounds. For the compounds β-mercaptoethylamine, cystein, histamine, ethylenediamine tetraacetic acid, ethylenediamine, salicylic acid, and glycine such data are available. The correlation coefficient between the percentage survival[1–3] and the $\log_{10}$ of the stability constants of the copper chelates[4] is 0·57. For a perfect correlation this would be 1·0 and for no correlation it would be 0.

If this mechanism is actually responsible for a considerable part of the protective action, it would suggest that the best protection would be afforded by compounds capable of tying up several of the co-ordination positions of a metal ion and thus use the chelate effect to enhance the protective action. The same substances which provide chemical protection should also provide protection against heavy-metal poisoning when injected before the heavy-metal. Furthermore, a wider class of materials should be capable of protective action than would be estimated on the basis of other theories of this action, such as that of mixed disulphide formation[7]. The requirements of a good protective agent appear to be very similar to those of a good chelating agent with some specificity for transition metals, especially copper. If this chelating action is important, the protective action found for ethylenediamine tetraacetic acid[3,8] should be found in enhanced measure with compounds such as 1,2-diamino*cyclo*hexane N,N,N′,N′-tetraacetic acid and diethylenetriaminepentaacetic acid.

An alternative explanation with somewhat similar consequences is one in which the catalytic effectiveness of the resulting chelate complexes in decomposing hydroxylonium or hydrogen peroxide is the basis of their action. This explanation could perhaps be reconciled with the theory of competition for hydroxylonium radicals of Bacq *et al.*[1,3].

Bacq and his co-workers briefly considered the possible importance of chelation but then dismissed it. Their chief evidence against it was obtained not on organisms but on polymethacrylic acid solutions, where the action of radiation is presumably analogous to that found with organisms. It seems very likely that the behaviour of organisms is considerably more complex. It is highly possible that there are two or more major types of radiation damage the cumulative effect of which is lethal. The control or elimination of one of these types may then reduce the cumulative damage to a level which is not lethal.

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BIOCHEMISTRY

Incorporation of α,ω-Dicarboxylic Acids as Side-chains into the Penicillin Molecule

WHILE a considerable number of biosynthetic penicillins have been obtained by adding different side-chain precursors to the culture medium, the incorporation of aliphatic dicarboxylic acids into the penicillin molecule does not as yet seem to have been reported.

When a high-yielding *Penicillium chrysogenum* strain of the Wisconsin family (*Wis* 51.20[1], subculture *c* isolated in this laboratory) was grown in shake flasks on a corn-steep lactose medium in the absence of a precursor acid (for conditions of growth see footnote of Table 1), the culture medium showed a slight activity against *Bacillus subtilis* (40–150 benzylpenicillin units per ml.) and a slightly higher activity (about 300 units per ml., benzylpenicillin as standard) against *Klebsiella pneumoniae*. Addition of the lower homologues of α,ω-dicarboxylic acids gave essentially similar results; but on addition of adipic acid or the higher homologues the antibacterial activity against *K. pneumoniae* considerably increased while that against *B. subtilis* was slightly decreased (Table 1).

The highest increases of anti-*Klebsiella* titres (up to ten-fold) were obtained on addition of adipic, pimelic and azelaic acid; the higher homologues gave somewhat lower titres. Similar results were obtained on a synthetic medium of the type developed by Jarvis and Johnson[2].

The penicillin formed after addition of adipic acid as side-chain precursor was isolated from larger quantities of culture media in purified form and

Table 1. Antibacterial Activity against *B. subtilis* and *K. pneumoniae* of Culture Filtrates of *P. chrysogenum* Strain *Wis* 51.20 c after 96 hr.

Precursor acid added		Final concentration in percentage of free acid‡	Activity against* *B. subtilis* (benzylpenicillin units/ml.)	Activity against* *K. pneumoniae*† (benzylpenicillin units/ml.)
None			185	280
Phenylacetic	(sodium salt)	0·2	1,700	1,600
Oxalic	(potassium salt)	0·7	200	330
Malonic	(sodium salt)	0·6	175	340
Succinic	,,	0·65	170	290
Glutaric	,,	0·7	170	300
Adipic	,,	0·8	100	1,000
Pimelic	,,	0·9	130	1,260
Suberic	(potassium salt)	0·95	150	1,040
Azelaic	,,	1·0	110	1,030
Sebacic	,,	1·1	100	900
1,10-decanedicarboxylic (C_{12})	,,	1·25	100	650
1,12-dodecanedicarboxylic (C_{14})	,,	1·4	100	800

Conditions of growth: Culture medium—lactose 3 per cent, glucose 0·5 per cent, corn-steep liquor 4 per cent, calcium carbonate 1 per cent, seeded with 10 per cent of a 48-hr.-old mycelial suspension grown on a corn-steep liquor (8 per cent) – dextrin (6 per cent) medium, *p*H adjusted to 5·4 with sodium hydroxide, from a suspension of conidia developed on barley moistened with the sporulating solution of Whiffen and Savage (ref. 4). 500-ml. Erlenmeyer shake flasks with 60 ml. of culture media were agitated at 25° C. on a rotary shaker (ref. 9), sulphite aeration rate 35 ml. oxygen per 100 ml. fluid per hr.

* Averages of two shake flasks which gave results in very good agreement with each other.

† The figures are benzylpenicillin units giving inhibition zones equal to those given by the undiluted broth.

‡ Added in two lots of 1–4 ml. each (according to the solubility), at 24 hr. and 72 hr., of an aqueous solution of the sodium or potassium salts in concentrations to give the above final concentration values.

shown to be (4-carboxy-*n*-butyl)-penicillin, that is, to contain the adipic acid side-chain.

The mycelium was grown at 28° C. in fermentors of 90 l. capacity[3] containing 50 l. of a culture medium consisting of 4 per cent corn-steep liquor, 3 per cent lactose, 0·5 per cent glucose and 1 per cent calcium carbonate. (Conditions of aeration and agitation: sparger aeration in fully baffled system, propeller blade 130 mm. diameter rotating at 420 r.p.m., air flow 50 l. per min. during first 24 hr., then increased to 80 l., overpressure 1 atm. One portion of 50 ml. of vegetable (mainly soy bean) antifoam oil containing 4 per cent 'Alkaterge', added at the beginning, and further portions of 50 ml. during the fermentation if and when required.) The culture medium was seeded with 10 per cent of a 48-hr.-old mycelial suspension grown in another 90-l. fermentor under the same conditions of aeration in a medium containing 6 per cent dextrin, and 8 per cent corn-steep liquor, *p*H adjusted to 5·4 with sodium hydroxide. The seed medium was inoculated with a suspension of conidia, developed on barley moistened with the sporulating solution of Whiffen and Savage[4].

Adipic acid in the form of its sodium salt was added in a 25 per cent aqueous solution in two lots of 800 ml., one at 24 hr. and the second at 72 hr., to give a final concentration of 0·4 per cent after each addition, that is, a total of 0·8 per cent. The fermentation was allowed to proceed for 96–120 hr., after which time the titre against *K. pneumoniae* had reached about 1,500 benzylpenicillin units/ml., while the titre against *B. subtilis* was about 150 units/ml. The filtered culture medium was percolated through a column of granular charcoal (20–60 mesh, *BO* grade, Farnell Carbons, Ltd., London) prepared as described by Abraham, Newton and Hale[5]. 1 vol. of charcoal was used for 2 vol. of medium. After washing with 1/10 vol. of water and then 1 vol. of 20 per cent acetone, the active substance was eluted with 2 vol. of 70 per cent acetone. The eluate containing about 70 per cent of the active substance with a specific activity of about 80 hydroxylamine units/mgm. using the method of Ford[6] for the determination of penicillin (the value of the pure compound is 1,500 units/mgm.) was concentrated about 20 times *in vacuo* in a climbing film still[7] at a temperature not above 40° C., and then lyophilized. There was a loss of activity of about 20 per cent in this process. The dry material from the lyophilization was extracted 3 times with 4 ml. of methanol for each gram of dry substance. 2 vol. of acetone were added to the methanolic solution and the mixture was centrifuged to eliminate precipitated material of low activity. The supernatant was concentrated to a small volume and 10 vol. of acetone were added to this. The supernatant contained a mixture of penicillins (about 40 per cent of the total activity) with a ratio of activity against *K. pneumoniae*/activity against *B. subtilis*, similar to that of benzylpenicillin, while the precipitate contained the remainder of the active substance with a much higher activity ratio and a specific activity of 300–350 hydroxylamine units per mgm. The precipitate dissolved in water was passed through a column of 'Dowex' 1 × 8, 50–100 mesh, in the chloride form. 1 ml. of resin was used for about 10,000 hydroxylamine units of active substance. The activity was eluted with 5 per cent sodium chloride at *p*H 7. The active fractions were lyophilized, and the dry product was extracted with three portions of 100 ml. of dry ethanol. The addition of 4 vol. of acetone to the ethanolic solution precipitated a product which was dried with acetone and ether.

The yield from the ion-exchange chromatography and the successive operations was about 50–60 per cent and the final product was 70–80 per cent pure. The active product was rapidly destroyed by penicillinase. It could not be extracted by any organic solvents at an acid *p*H. In paper chromatography on Whatman No. 1 at 5° C., in *n*-butanol/ethanol/water, 40 : 10 : 50 v/v/v, it remained at the origin; in 70 per cent aqueous *n*-propanol it had an $R_F = 0{\cdot}35$ while benzylpenicillin had an R_F of 0·75. On paper electrophoresis (0·1 *M* phosphate buffer, *p*H 6·8, applied potential 25 V./cm.) it had a mobility about twice (20 cm. per hr.) that of benzylpenicillin (10·5 cm. per hr.). After hydrolysis for 2 hr. at 100° C. in 2·5 *N* hydrochloric acid, subsequent ether extraction and evaporation of the ether extracts, crystals appeared in a yield of 60 per cent of the theoretical

Table 2. Comparison of Antibacterial Properties of Benzylpenicillin and (4-Carboxy-*n*-butyl)-penicillin*

	Benzylpenicillin (μgm./ml.)	4-Carboxy-*n*-butyl penicillin (μgm./ml.)†
Bacillus subtilis	0·027	0·52
Micrococcus flavus	0·30	1·57
Sarcina lutea	0·01	0·65
Staphylococcus aureus (Oxford)	0·06	1·57
Streptococcus pyogenes	<0·4	>32
Klebsiella pneumoniae	18	18
Salmonella typhimurium	10·84	4
Salmonella typhi	2·4	5
Escherichia coli	36·14	>67

* Serial broth dilution technique: 0·1 ml. of a 1 : 80 dilution of a 24-hr. broth culture of the respective bacteria was inoculated into 9·9 ml. broth of the following composition—meat extract 1·0 per cent, sodium chloride 0·5 per cent, peptone 1·0 per cent, glucose 0·05 per cent.

† Corrected for impurities.

amount expected, which were identified as adipic acid by melting and mixed melting point as well as by their paper chromatographic behaviour in two solvents (ethanol/conc. ammonia/water, 80 : 4 : 16, v/v/v, and *n*-butanol/acetic acid/water, 40 : 10 : 50, v/v/v).

A comparison of the minimal growth-inhibiting concentrations of (4-carboxy-*n*-butyl)-penicillin and benzylpenicillin against a range of different Gram-positive and Gram-negative bacteria is given in Table 2. From the results it is clear that (4-carboxy-*n*-butyl)-penicillin is very similar in its activity to cephalosporin *N* as reported by Heatley and Florey[8], that is, it has a much lower activity than benzylpenicillin against the Gram-positive and about the same order of activity against the Gram-negative bacteria. The lowering of the activity against Gram-positive bacteria is therefore not a specific effect of the α-amino group in the α-aminoadipic side-chain, present in cephalosporin *N*, but can be obtained by the introduction of the simple adipic acid side-chain or its higher homologues into the penicillin molecule, that is, by the presence of an extra free carboxylic group. A *K. pneumoniae* strain made resistant to benzylpenicillin by subculturing in the presence of subminimal inhibitory concentrations of benzylpenicillin (it grew eventually in 6,000 units per ml.) was also found to be resistant to (4-carboxy-*n*-butyl)-penicillin. Skin tests of (4-carboxy-*n*-butyl)-penicillin in seven highly benzylpenicillin-allergic subjects (carried out by Drs. R. Maffei and E. Errigo of the University of Rome) have shown that the compound does not elicit allergic reactions.

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Effect of Cardiac Glycosides and Inorganic Ions on Binding of Serotonin by Platelets

The presence of high concentrations of serotonin (5-hydroxytryptamine) in blood platelets has stimulated many studies on the mechanism of the uptake of the amine by these cells[1–5]. This process can readily be studied *in vitro* employing platelets suspended in plasma or in saline solutions, and there is now considerable evidence that this process is one of active transport[6].

A previous study from this laboratory[7] employing platelets suspended in isotonic sodium chloride indicated that inorganic phosphate is required in order to obtain maximum binding of 5-hydroxytryptamine. This communication presents a more detailed study of the factors affecting the binding of 5-hydroxytryptamine by blood platelets in well-defined media. By controlling the *p*H, it appears that the active transport mechanism can be studied independently of other mechanisms that may be functioning. In such a system inorganic phosphate and potassium are required for maximal uptake. The cardiac glycosides are known to inhibit the active transport of cations by several tissues[8] as well as the uptake of iodide by isolated thyroid slices[9], and it seemed of interest to test their ability to inhibit the transport of other substances. Both the cardiac glycosides and reserpine, which has previously been shown to inhibit the uptake of 5-hydroxytryptamine by platelets[4], inhibited this system.

Human platelets were isolated according to the method of Dillard *et al.*[10], washed as described previously[7] and resuspended in saline. When platelets were incubated with 5-hydroxytryptamine the concentration of the latter in the platelets continued to increase for approximately 90 min. Incubations were carried out for 1 hr. at 37° C., during which time uptake was nearly linear. The platelets were re-isolated, washed and assayed for both protein[11] and 5-hydroxytryptamine[12]. Results are expressed as μgm. of 5-hydroxytryptamine per mgm. of platelet protein.

A marked effect of *p*H on the relationship between the uptake of 5-hydroxytryptamine and its concentration in the medium was observed. At *p*H 5·7 with potassium and phosphate present (Fig. 1) the system became saturated at low concentrations of amine (less than 5 μgm./ml.). At higher *p*H, however, another mechanism appeared, involving a more nearly linear dependence of 5-hydroxytryptamine accumula-

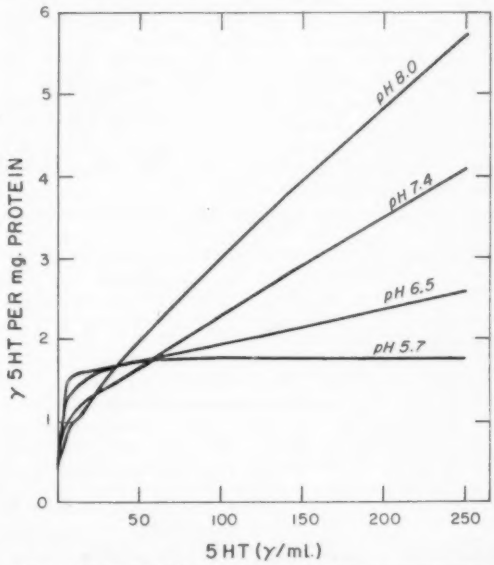

Fig. 1. The effect of *p*H and 5-hydroxytryptamine concentration on platelet uptake of the latter. The incubation mixture contained 1 ml. of platelet suspension (about 1 mgm. protein) in 0·9 per cent sodium chloride, 5-hydroxytryptamine (dissolved in 0·9 per cent sodium chloride and adjusted to *p*H 5·7), 50 μmoles phosphate buffered at the appropriate *p*H, and 0·9 per cent sodium chloride to make up to a final volume of 2 ml.

Table 1. EFFECT OF POTASSIUM AND PHOSPHATE ON UPTAKE OF 5-HYDROXYTRYPTAMINE AT *p*H 5·7
(μgm. of 5HT per mgm. of platelet protein over control*)

System	Experiment No. (μgm./mgm. protein) 1	2	3	4	Average percentage of maximal stimulation (γ)
A Platelet + 5HT + K^+ (45 μmoles) + PO_4^{3-} (50 μmoles)	0·62	0·84	1·17	0·62	100
B Platelet + 5HT + PO_4^{3-} (50 μmoles)	0·22	0·40	0·51	0·34	46
C Platelet + 5HT + K^+ (50 μmoles)	0·43	0·49	0·76	0·48	68

* Values are those over a control in which platelets were incubated only with 5HT (30 μgm.) and 0·9 per cent sodium chloride to make up to a final volume of 2 ml.
The incubations contained 1 ml. of platelet suspension in 0·9 per cent sodium chloride (about 1 mgm. protein), 30 μgm. 5HT (0·3 ml.) in 0·9 per cent sodium chloride and 0·9 per cent sodium chloride to make up to a final volume of 2 ml. 0·1 ml. of the following solutions was added. System *A*, phosphate buffer *p*H 5·7 (0·5 *M* with respect to phosphate containing 450 μmoles/ml. of K^+ and 90 μmoles/ml. Na^+). System *B*, 0·5 *M* sodium dihydrogen phosphate (adjusted to *p*H 5·7). System *C*, 0·5 *M* potassium chloride.
5HT = 5-hydroxytryptamine.

tion on the amine concentration. This relationship between uptake and concentration suggests that a diffusion mechanism predominates at the higher *p*H value.

Platelets suspended in saline alone, at *p*H 5·7, were able to bind exogenous 5-hydroxytryptamine. However, the uptake was much greater when potassium and phosphate were added. As shown in Table 1 both phosphate and potassium were required for optimal uptake. Of the total attainable stimulation of 5-hydroxytryptamine uptake approximately 45 per cent could be produced by addition of phosphate alone and 70 per cent by potassium alone. Maximal effects were obtained with as little as 10 μmoles potassium and 1 μmole phosphate.

As shown in Table 2 both reserpine and oubain inhibited the uptake of 5-hydroxytryptamine in this system. However, the inhibition observed was only on the potassium and phosphate effects. The uptake of 5-hydroxytryptamine which occurred when the platelets were suspended in saline alone (usually a 2–3 fold increase over the control) was not affected by these drugs. The cardiac glycosides used routinely were oubain, digitoxin and strophanthin potassium, all of which had equal activity at 10^{-5} *M*. None of the glycosides inhibited at 10^{-7} *M*.

Table 2. EFFECT OF COMPOUNDS ON PLATELET UPTAKE OF 5-HYDROXY TRYPTAMINE AT *p*H 5·7
(Increase in μgm. of 5HT per mgm. of platelet protein over endogenous*

System	Experiment No. 1	2	3	4	5	6
A Platelet + 5HT	0·58	0·97	0·74	0·52	0·84	0·53
B Platelet + 5HT + K^+ + PO_4^{3-}	0·96	1·36	1·22	1·03	1·09	1·62
C Platelet + 5HT + K^+ + PO_4^{3-} + oubain	0·65	1·12	0·91	0·46	0·78	0·87
D Platelet + 5HT + K^+ + PO_4^{3-} + reserpine	0·53	1·08	—	0·52	0·70	0·60
E Platelet + 5HT + oubain	0·64	1·18	0·78	—	0·84	0·61
F Platelet + 5HT + reserpine	0·69	1·10	0·63	—	0·92	0·42

*The endogenous values in the experiments to be described in this report varied generally between 0·1–0·4 μgm. 5HT per mgm. of platelet protein.
Each incubation mixture contained 1 ml. of platelet suspension (about 1 mgm. of protein) and 30 μgm. of 5HT (0·3 ml.). 50 μmoles of phosphate, 45 μmoles of K^+, oubain (10^{-5} *M* final concentration), and reserpine (10^{-5} *M* final concentration) were added where indicated. The final volume was made up to 2 ml. with 0·9 per cent sodium chloride.
5HT = 5-hydroxytryptamine.

Unlike the steroids and reserpine, 10^{-3} *M* fluoride inhibited almost completely the uptake of 5-hydroxytryptamine whether or not potassium and phosphate were present. Since the uptake of 5-hydroxytryptamine by the platelets proceeds under anaerobic conditions and is not inhibited by 2,4-dinitrophenol, it has been suggested that glycolysis supplies the energy required for this process. The inhibition observed with fluoride supports this postulate. The mechanism of inhibition by the glycosides remains unclear. However, in mammalian erythrocytes, which presumably derive energy for the transport of cations from glycolysis, the cardiac glycosides are also effective inhibitors of the transport of sodium and potassium[13] but do not affect the synthesis of adenosine triphosphate or the metabolism of the phosphorylated intermediates in glycolysis[14,15].

It is of interest that under more alkaline conditions (*p*H 8) where a diffusion type mechanism appears to predominate, reserpine, the cardiac glycosides and fluoride had much less effect on the accumulation of 5-hydroxytryptamine by platelets.

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Effect of Streptomycin on the Incorporation of Tyrosine labelled with Carbon-14 into Protein of *Mycobacterium* Cell Fractions *in vivo*

STREPTOMYCIN inhibits the incorporation of tyrosine labelled with carbon-14 into the protein of a cell-free system isolated from *Mycobacterium friburgensis*, but the degree of inhibition is less when intact cells are incubated in a culture medium[1].

We assume that streptomycin does not inhibit the incorporation into the protein of all cell fractions, and investigated, therefore, the rate of labelling of whole cell protein and various cell fractions on incubation of intact cells with labelled tyrosine.

The bacteria were washed and resuspended in a Sauton medium at a concentration of 200 mgm. wet

Table 1. Effect of Streptomycin on the Incorporation of Labelled Tyrosine into Protein of *Mycobacterium friburgensis* Cell Fractions *in vivo*

Experiment No.	Incubation time (min.)	c.p.m./mgm. protein							
		Whole cells		Fraction *A*		Fraction *B*		Fraction *C*	
		–	+ Streptomycin	–	+ Streptomycin	–	+ Streptomycin	–	+ Streptomycin
1	30	219	165	132	128	192	134	214	102
2	10	122	68	65	65	12	8	126	31
2	30	150	80	112	122	35	15	139	60
3	10	126	55	26	29	55	9	27	8

weight per ml. 5 ml. cell suspension were incubated for 30 min. with shaking at 37° C. in the presence of 5 μc./ml. labelled DL-tyrosine (1 μc. = 17×10^6 c.p.m.) with or without addition of 100 μgm./ml. streptomycin. The cells were washed twice with 20 vol. of ice-cold distilled water, and resuspended in 6 ml. buffer[2]. 1 ml. was removed to determine incorporation into total cell protein, to the remaining 5 ml. cell suspension 0·8 gm. alumina was added, and the cells were disintegrated by grinding in a chilled mortar for 20 min. The suspension was centrifuged at 1,200*g* for 10 min. (the sediment-fraction *A*). The supernatant was centrifuged in a Spinco preparative centrifuge at 105,000*g* for 60 min. to yield a particle fraction (*B*) and a clear supernatant (*C*).

The protein of the whole cells and of the fractions *A*, *B* and *C* was isolated and the radioactivity determined. The results of the experiments are shown (Table 1).

They show that the extent of inhibition differs markedly in the fractions, being greatest in the fraction *C* (which corresponds to our 'cell-free' system)[1], while no inhibition was found in fraction *A* (cell wall and membrane fragments).

These results suggest that streptomycin does not inhibit the protein synthesis of the cell wall.

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Peptides obtained from Partial Hydrolysis of Decalcified Human Dentin Collagen

From *in vitro* studies of mineralizing connective tissues, it has been suggested that the arrangement of polarized protein macromolecules in the submicroscopic collagen fibrils may be an important factor in determining whether suitable 'nucleation centres' for hydroxyapatite crystals will be present[1]. The banded regions of natural and reconstituted collagens are thought to contain relatively high proportions of the bulky basic and acidic amino-acid residues, thus suggesting that the amino-acid residues in collagen are not randomly distributed. This view has already been verified for soft tissue procollagen by an analysis of peptides obtained by enzymatic hydrolysis[2]. Since soft and hard tissue collagens differ markedly from each other with respect to the availability of their ε-amino-groups to dinitrofluorobenzene[2], as well as in their solubility behaviour[1] and internal molecular structure[4], it is of interest to compare the composition, amino-acid sequence and structure of highly polar peptides obtained from both types of collagen.

Small pieces of dentin from the roots of freshly extracted adult human anterior teeth were demineralized by soaking in 1 *N* hydrochloric acid for 24 hr. at 4° C. The demineralized dentin collagen was washed with cold 0·16 *N* potassium chloride solution for 24 hr., rinsed with distilled water, and allowed to dry in air. Weighed samples (0·1 gm.) were heated in distilled water (20 ml.) in a sealed tube at 110° C. for 16 hr. The resulting hydrolysate was evaporated to small volume on a steam bath, and then separated into 4 ninhydrin-positive bands labelled *A*, *B*, *C* and *D*, by low-voltage electrophoresis for 2 hr. on Whatman No. 1 filter paper using 1·2 *N* acetic acid as solvent. Migration occurred towards the cathode. The positions of the amino-acids and peptide spots were visualized by spraying the dried chromatograms with 0·1 per cent ninhydrin in acetone, and heating at 105° C. for 2 min. Each band was eluted with 6 *N* hydrochloric acid (redistilled over stannic chloride), and hydrolysed for 16 hr. in a sealed tube at 105° C. Portions of the hydrolysate, as well as the mixture before acid hydrolysis, were examined by two systems of paper chromatography, employing butanol/acetic acid/water (4 : 1 : 5) and phenol/water (7 : 3) as solvents.

It was observed that the main products of the water hydrolysis of dentin collagen were peptides *A*, *B*, *C* and *D* together with minimal amounts of free amino-acids. The peptides *A*, *B*, *C* and *D*, labelled in order of increasing mobility towards the cathode, differed markedly from each other in their qualitative amino-acid composition. Band *B* was typical of the composition of the intact protein, all the amino-acids normally found in mammalian collagen being present. Band *A* resembled band *B*, except that hydroxyproline was absent. In band *C*, hydroxyproline, proline and phenylalanine were all absent. Band *D* contained 7 amino-acids—lysine, hydroxylysine, glycine, alanine, arginine, glutamic and aspartic acids. This highly polar band was further investigated and found to be homogeneous in the chromatographic systems used, and on electrophoresis at different *p*H values. By means of Sanger's dinitrofluorobenzene technique[5] it was shown that alanine was in the N-terminal position of the intact peptide *D*. Under the experimental conditions cited, the results were reproducible.

These preliminary results show that the distribution of amino-acids in dentin collagen is non-random and resembles to some extent that found in soft tissue procollagen[3]. The amino-acid sequence of the peptides, as well as their relative efficiency as 'seeding' agents for hydroxyapatite crystals, are currently

being investigated, and the results will be reported at a later date.

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Inactivation of Erythropoietin by Neuraminidase and by Mild Substitution Reactions

Erythropoietic activity in the plasma of rabbits made anæmic with phenylhydrazine is associated with mucoproteins[1–4]. It has been shown that the activity is destroyed by proteolytic enzymes[1,2,5]. Rambach *et al.*[6] have found as well as us that heating at 100°, *p*H 2, for 1 hr. destroys the activity, a treatment which, in the case of guinea pig α_1-acid glycoprotein[7], selectively removed sialic acid. In spite of the mildness of this treatment it is possible that other bonds (sugar, amino-acid) were broken. We have now been able to show that exposure to neuraminidase[8] completely inactivates the mucoprotein.

An acid glycoprotein (15·6 per cent sialic acid) has been regarded as erythropoietin by Rambach *et al.* We[2] have pointed out that it is probable that only a small proportion of the concentrates yet prepared is the active erythropoietic material. Recent work by Campbell *et al.*[11,12] with sheep plasma has strengthened this conclusion. Their most active preparation is lower in sialic acid.

The cold, neutralized filtrate from heat-coagulated anæmic rabbit plasma[2] is mixed with 1·5 vol. of absolute ethanol and allowed to stand at 5° overnight. The inactive precipitate is removed by centrifugation in the cold and the supernatant mixed with an equal volume of absolute ethanol. After standing overnight, the precipitate is collected in the cold and dried in a desiccator. It is extracted with 20 parts of 0·01 *M* sodium acetate, *p*H 4·8, and the soluble portion (about 85 per cent) is dialysed against distilled water. The dialysand is lyophilized. 60–120 mgm. of a very soluble white powder are obtained from 1 litre of plasma. We will refer to this preparation as *LD*.

When *LD* is applied to a column of 'DEAE' cellulose (1 gm.) equilibrated in 0·01 *M* sodium acetate, *p*H 4·8, 15–30 per cent of protein emerges as a front, 10–20 per cent as another peak with 0·05 *M* sodium chloride as eluant. Both are erythropoietically inactive. Elution with 0·1 *M* sodium chloride yields a peak containing all the activity and 50–60 per cent of the protein. It again gives a single peak when rechromatographed on a similar column. Its properties are listed in Table 1.

A potent human urinary ultrafiltration residue[9], a gift of Dr. Van Dyke, was applied to a 'DEAE' column as above. 0·25 *M* sodium chloride had to be used for elution of the activity. Paper electrophoresis at *p*H 8·6 and 4·5 showed it to be similar to *LD* (mobility like an acid α_1 protein). Similar behaviour is shown by erythropoietin obtained from unboiled sheep plasma before its final purification[11,12].

Table 1

	Buffer	*p*H	Ionic strength	Mobility × 10^5 cm.^{2} volt sec.
Moving boundary electrophoresis:	Sodium barbitarate	8·75	0·1	− 5·40
	Sodium acetate	4·25	0·1	− 2·92
	Glycine hydrochloride	2·34	0·1	+ 0·79
Isoelectric point		2·75		
E(1 per cent, 1 cm.) 280 mμ		7·0		
Protein bound hexose (orcinol)		9·4%		
Hexosamine (Elson-Morgan, Rimington)		9·0%		
Sialic acid (resorcinol hydrochloride, Svennerholm (ref. 17) 15 min., 100° C.)		13–14%		
Methyl pentose		0·66–0·80%		

Neuraminidase treatment. (*a*) 15 mgm. *LD* in 2·4 ml. 0·05 *M* calcium acetate, *p*H 6, were incubated with about 30,000 units of neuraminidase[8] at 35°. After 1 hr., 0·1 ml. of 1 *M* sodium citrate and 10 ml. water were added, and the mixture boiled for 5 min. It was erythropoietically inactive. No loss of activity was found in a control experiment without enzyme added. (*b*) In similar experiments the reaction was stopped by sodium citrate alone without boiling after 10 min. and 1 hr. respectively, and each reaction mixture was then dialysed against 10 vol. of water. In both cases practically all the sialic acid of *LD* was found in the dialysate, and both dialysates and dialysands were erythropoietically inactive. Peptide bond hydrolysis (increase in amino-nitrogen) was insignificant and probably did not occur.

A number of relatively mild substitution reactions were carried out with *LD*:

(1) *Iodination* (ref. 13). To 10 mgm. *LD* in 0·4 ml. 0·35 *M* sodium phosphate *p*H 7·6, increments of 0·1 *N* iodine were added at room temperature. 0·07–0·13 ml. were taken up in 3½ hr.

(2) *Acetylation* (ref. 14). To 10 mgm. *LD* in 0·2 ml. of a solution of 11·9 gm. sodium acetate in 20 ml. water (*p*H = 9·25) 0·03 ml. of acetic anhydride was added over a period of 1 hr. at 0°.

(3) *Formaldehyde* (ref. 15). 10 mgm. *LD* in 0·1 ml. 0·35 *M* sodium phosphate *p*H 7·6 and 0·01 ml. 40 per cent formaldehyde were allowed to stand for 20 hr. at 8°. Excess formaldehyde removed *in vacuo*.

(4) *Esterification* (ref. 16). To 10 mgm. *LD* in 0·4 ml. methanol at −15° 0·02 ml. of thionylchloride were added over 3 hr. Methanol was removed *in vacuo*.

All four of the above reaction mixtures were erythropoietically inactive. (Assayed by iron-59 uptake in fasted rats[3,18].)

The enzymatic loss of erythropoietic activity indicates that bound sialic acid is an essential part of the molecule. It remains to be seen whether the high sensitivity of the molecule to substitution reactions carried out under conditions too mild for hydrolysis is related only to the sialic acid moiety or other sugar or amino-acid constituents of the active mucoprotein.

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Co-factors in *Hevea brasiliensis* Latex Serum

IT has recently been shown[1] that the incorporation of mevalonic acid into rubber by an enzyme system in *Hevea* latex *in vitro* is not dependent on the addition of such co-factors as are known to be necessary for the incorporation of mevalonic acid into squalene. Such co-factors include adenosine triphosphate, cysteine and/or glutathione in the case of cell-free preparation of rat liver[2], although Amdur, Rilling and Bloch[3] found that whereas adenosine triphosphate was necessary with a soluble extract of bakers' yeast, no reducing agent requirement was demonstrated. Popjak and Gore show that under anaerobic conditions the reducing agent is not necessary but it is essential for the formation of squalene aerobically. Tchen[4] has demonstrated that the purified enzyme 'mevalonic acid-kinase' responsible for part of the pathway, namely, the phosphorylation of mevalonic acid, requires the presence of any of the four nucleoside triphosphates (including cytidine triphosphate). He shows also that the enzyme is inhibited by chloromercuribenzoate.

The present communication is to record the detection and assay of di- and tri-phosphates of adenosine and cytidine and of cysteine and reduced glutathione in samples of freeze-dried fresh latex sera obtained from Malaya. This material[5] would be expected to be equivalent to the serum from fresh latex 1–2 hr. after tapping, so far as soluble co-enzyme content is concerned. Details of these investigations will be published elsewhere; but it is thought of interest to record that such samples of normal latex sera examined contain approximately 0·7 micromole of glutathione and 0·4 micromole of cysteine per ml. and at least 0·0005 per cent nucleoside diphosphate in the form of adenosine diphosphate and cytidine diphosphate (predominantly as adenosine diphosphate), and almost the same amount of nucleoside triphosphate (predominantly as cytidine triphosphate).

It would be of interest to determine the influence of —SH inactivating agents on both the aerobic and anaerobic incubation of mevalonic acid labelled with carbon-14 in fresh latex to establish the similarity or otherwise of the general reductive mechanism in the two instances.

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Isolation of the Neurotoxic Component of the Venom of the Sea Snake, *Enhydrina schistosa*

THE sea snake, *Enhydrina schistosa*, is widely distributed in the coastal regions of the Far East and many fatalities from its bite are reported[1]. The *LD*50 of the venom for a wide variety of laboratory mammals is in the region of 50–100 μgm./kgm. body-weight. On this basis it is four times as toxic as the venom of cobra. Venom yields per bite of up to 55 mgm. of dried venom have been obtained from captive snakes. If humans are of equal susceptibility to laboratory animals, up to 15 *LD*50 for humans may be expected from one bite. At present there is no supply of antivenom available for treatment, and it is doubtful if the titres obtainable by even the best available preparative methods would be sufficient to allow the antivenom being administered in a life-saving dose. The separation of the neurotoxic component of this venom is important from the point of view of analysing its biochemical actions and enhancing its antigenic properties.

Carey and Wright[2] have shown that the whole venom consists of at least three antigenic components. The neurotoxic component is the most electropositive (*p*H 6·8 in agar gel) and will pass through a cellulose but not a nylon dialysis sac.

In the present investigation it has been found that after repeating the cellulose sac dialysis against large volumes of distilled water approximately 1 per cent of the initial toxicity remains inside the sac. This may be due to a different toxic component or to adsorption of traces of dialysable component on to non-dialysable material. However, it is unlikely to contribute significantly to the toxicity of the whole venom. Immunoelectrophoresis of the cellulose sac dialysate using antiserum to the whole venom shows only one main precipitation line. However, this has since been shown to consist of at least two antigenic components by varying the relative concentrations of venom and antiserum in the immunoelectrophoresis. When the dialysate was placed on a carboxy methyl cellulose column and eluted with *p*H 6·6 buffers of graded molarities from 0·06 to

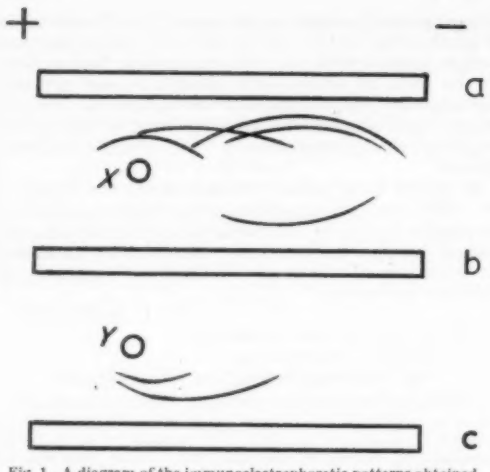

Fig. 1. A diagram of the immunoelectrophoretic patterns obtained with *Enhydrina schistosa* venom at *p*H 6·8 in phosphate buffer and electrophoresed for 3 hr. at 4 volts/cm. *a*, Antiserum to the whole venom in channel; *b*, antiserum to the toxic fraction in channel; *c*, antiserum to the whole venom in channel; *X*, whole venom at origin; *Y*, whole venom mixed with excess antiserum to the toxic fraction at origin

0·08 *M*, a further purification of the toxic component was obtained. Toxicity equivalent to 80–100 per cent of that of the initial sample was recovered in the eluant in one sharply defined zone. Immunoelectrophoresis of this sample using antiserum to the whole venom gave one precipitation line only over a range of relative concentrations of venom and antiserum. When whole venom is mixed with excess antiserum prepared against the toxic fraction only, the supernatant is non-toxic but still contains antigenic material as demonstrated by immunoelectrophoresis. The immunoelectrophoretic patterns of this supernatant material compared with that of the whole venom using antiserum to the whole and fractionated venom are shown in Fig. 1. An antiserum to this one fraction may therefore be as useful therapeutically as an antiserum to the whole venom. There was, however, no demonstrable advantage in using the purified toxic fraction as the antigen in the preparation of a protective serum.

Conflicting reports have appeared in the literature regarding the importance of a lecithinase in the toxicity of some venoms[2–5]. For demonstrating lecithinase activity we incubated the venom with egg yolk and then tested the solution for hæmolytic activity. After dialysis using a 'Cellophane' membrane, more than 90 per cent of the lecithinase activity of the whole venom could be retained inside the sac while more than 90 per cent of the initial toxicity passed through. This distinction between the two factors is further supported by the finding that the toxicity of the whole venom is more readily destroyed by heating than is its lecithinase activity.

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Transglucosidase Activity in *Acetobacter xylinum*

Cells of *Acetobacter xylinum* are known to produce cellulose. This synthesis has been demonstrated in resting fresh and lyophilized cells[1,2]. Enzymic synthesis of cellulose by preparations from *A. xylinum* has been shown with the aid of tracer techniques[3,4] and electronmicrography[5].

Glaser's experiments[4] with uridine diphosphoglucose labelled with carbon-14 in the glucose moiety demonstrate the incorporation of glucose into cellulose via uridine diphosphoglucose:

$$\text{uridine diphosphoglucose} + (\text{glucose})_n \longrightarrow \text{uridine diphosphate} + (\text{glucose})_{n+1}$$

No experimental evidence has been presented for the reverse reaction, that is, the formation of uridine diphosphoglucose from uridine diphosphate and soluble cellodextrins. If the reaction is reversible, cellodextrin molecules may serve both as donors and acceptors of glucose units; the donors thereby become shorter, while the acceptors increase in length. Most likely there is a certain probability for obtaining some alkali-insoluble chains of high molecular weight.

In the present experiments a net increase of alkali-insoluble carbohydrate material was found upon the addition of uridine diphosphate and soluble cellodextrins (the soluble cellodextrins were made by a modified procedure of the method given by Zechmeister *et al.*[6]) to a preparation from *A. xylinum*.

Pellicles of *A. xylinum* (N.C.I.B.8246) were freed from cellulose by the method of Hestrin and Schramm[3], the cells were ruptured, with careful *p*H control, with an ultrasonic disintegrator, and centrifuged to eliminate remaining cells and cell-debris. In some experiments the cell-free extract was used as such. Attempts were made to fractionate the extracts by collecting the precipitates at 19,000*g* and 35,000*g*, and resuspending the pellets in 0·1 *M* *tris*(hydroxymethyl)aminomethane buffer of *p*H 8. The reaction was carried out in centrifuge tubes kept in an evacuated container at 30° C. After 20 hr. NaOH was added to a final concentration of 1 per cent, and the tubes were heated in a boiling water-bath for

Table 1

Experiment	Additions	Alkali-insoluble material formed. As: Hexose (μgm.)	Net increase (per cent)
1	None	180	—
	Uridine diphosphoglucose	200	11
	Uridine diphosphoglucose, cellodextrins	320	78
	Cellodextrins	220	22
2	None	8	—
	Uridine diphosphoglucose	12	50
	Uridine diphosphoglucose, cellodextrins	17	112
	Cellodextrins	14	75
3	None	70	—
	None	68	—
	Uridine diphosphate, cellodextrins	188	172
	Cellodextrins	118	71
	Uridine diphosphate, cellodextrins, cellobiose	184	167
	Cellodextrins, cellobiose	94	36
	Uridine diphosphate, cellobiose	56	—
	Cellodextrins, unincubated	64	

Each tube contained 1 ml. enzyme preparation of *p*H 8, plus additions as given, in the following amounts: uridine diphosphoglucose, 8·5 μmoles; uridine diphosphate, 9·7 μmoles; cellobiose, 59 μmoles; cellodextrins, 15–20 mgm.; total volume per tube, 2 ml. Different enzyme preparations were used in the three experiments. In 1, the pellet from 19,000*g*; in 2, the pellet from 35,000*g*; and in 3, the non-fractionated extract.

5 min. and then centrifuged. The precipitates were washed twice with water and dried. Hexose was determined by the anthrone method[7]. The results are presented in Table 1.

These results indicate the presence of transglucosidase activity in the preparation from *A. xylinum*. The insolubilization of soluble cellodextrins through the action of transglucosidase will from statistical reasons be expected to give low yields of high molecular weight compounds. Further work is in progress to purify to some extent the enzyme system in order to investigate the nature and specificity of co-factors in the reaction.

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Preparation of Crystalline Cytochrome$_{553}$ from *Porphyra tenera*

PARTIAL purification of a cytochrome component from *Porphyra tenera*, a component which has absorption peaks similar to that of cytochrome *c* or c_1 was first reported by Yakushiji[1]. Recently, Katoh and Takamiya (ref. 2 and Katoh, S., and Takamiya, A., personal communication) succeeded in obtaining a crystalline preparation of this component by means of diethylaminoethyl-cellulose column chromatography and reported the redox-potential of this and other components in marine algae. We also have crystallized the cytochrome by means of a cation-exchanger at *p*H 5·0. Since the diethylaminoethyl-cellulose column procedure is possible at *p*H 7·0, this procedure may be better; our method can be used to purify not only basic enzyme proteins, but also those that are stable in acidic *p*H.

About 700 gm. of the algae dried by exposure to the Sun was suspended in 12 l. of water and kept for 3 days at 15°. The suspension was squeezed through a cheese-cloth and about 11 l. of reddish-violet extract obtained. To this extract solid ammonium sulphate was added to obtain a concentration of 50 per cent saturation and the resulting precipitate, which contains almost all phycobilin, was removed from the extract by filtration. To recover the cytochrome from the precipitate, the precipitate was dissolved in 1 l. of 0·05 *M* disodium hydrogen phosphate and the above fractionation was repeated with ammonium sulphate. Both filtrates were combined and the cytochrome precipitated by further addition of ammonium sulphate to raise the concentration to 100 per cent saturation. The precipitate, collected by filtration with the aid of 'Celite', was dissolved in about 500 ml. of 0·05 *M* disodium hydrogen phosphate and 1 per cent of rivanol added drop by drop to the solution until fibrous substances no longer formed. These substances were removed by filtration and the filtrate fractionated with ammonium sulphate to obtain a fraction between 80 and 100 per cent saturation. By repeating this fractionation twice, an excess of rivanol was removed. The precipitate was dissolved in 50 ml. water and dialysed against 2 l. of 0·02 *M* sodium acetate buffer of *p*H 5·0 overnight at 5°. After the dialysis, the solution was centrifuged to remove dark brown precipitates which appeared during the dialysis. The clear supernatant fluid was then charged on the top of column (1 × 50 cm.) of a cation-exchanger ('Amberlite *CG*–50', type II) buffered at *p*H 5·0 with the same buffer. The column adsorbed the cytochrome and was washed with 2 l. of the same buffer and then 2 l. of 0·1 *M* sodium acetate buffer, *p*H 5·0. These washings eluted some of the impurities and modified cytochrome. The adsorbed cytochrome was then eluted with 0·1 *M* sodium acetate buffer of *p*H 5·8. About 1 l. of eluate contained cytochrome which was concentrated by treatment with polyethyleneglycol[3]. This chromatography was carried out in the oxidized state, though chromatography is also possible in the reduced state at *p*H 5·0. In the latter case, however, an affinity of the cytochrome for resin is less than that of the oxidized state, so that the elution process was performed with 0·02 *M* buffer at *p*H 5·0 and it is necessary to repeat the chromatography twice in order sufficiently to remove the impurities. To the eluate solid ammonium sulphate was added to precipitate the cytochrome completely. The cytochrome was collected by centrifugation and dissolved in a minimum amount of water. Insoluble material is removed by centrifugation, and solid ammonium sulphate was added to the supernatant fluid by a small glass rod in several portions until small turbidities appeared. The solution was kept in a tap-water bath and the turbidity slowly increased. Within 2–3 hr., the crystals began to appear and almost all the cytochrome crystallized as needles within 1 day. The crystals were collected by centrifugation, washed with 90 per cent saturated ammonium sulphate solution, dissolved in a minimum amount of water, and then recrystallized as above.

Fig. 1 shows the absorption spectrum of a once-recrystallized preparation dissolved in 0·1 *M* phosphate buffer of *p*H 7·4. The α-band of the cytochrome shows its peak at 553 mμ and a shoulder at about

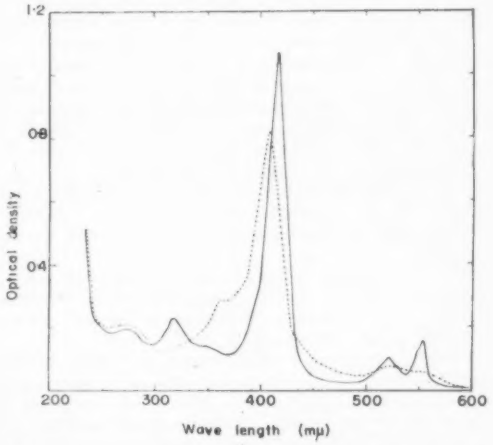

Fig. 1. Absorption spectrum of cytochrome$_{553}$ from *Porphyra tenera*. The crystals were dissolved in 0·1 *M* phosphate buffer of *p*H 7·4 and dialysed against the same buffer. Measurements are carried out by a Cary spectrophotometer Model 14 by using a 1-cm. light-path cuvette. The dotted line shows the oxidized form and the solid line the reduced form after the addition of sodium borohydride

550 mμ. However, other peaks are similar to that of beef cytochrome *c* or c_1. The absorption peaks of pyridine hæmochromogen at a high *p*H are similar to that of cytochrome *c* and the α-peak has no longer a shoulder. The hæm cannot be split from the protein moiety by the usual acid–acetone method (1 ml. 20 per cent hydrochloric + 100 ml. acetone). From these results it appears that the hæm of the cytochrome belongs to hæm *c*. The reduced cytochrome is not autoxidizable and is oxidized by cytochrome a_2 purified from *Pseudomonas aeruginosa*[4], but not by mammalian cytochrome *a*[5].

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ANIMAL PHYSIOLOGY

Withdrawal of Inhibition of Mitosis as a Mechanism influencing Normal and Pathological Growth

Experimental evidence has recently been given[1] of the likelihood that wound-healing occurs not from the stimulation of wound hormones directly, as has been thought for many years, but by the withdrawal of inhibiting influences as a result of cell damage.

Several experiments performed in this laboratory using regenerating liver of rats and guinea pigs have demonstrated that corticosteroids act as inhibitors to growth, and circumstances in which these substances are withdrawn or reduced have simulated a 'growth hormone effect'. From these observations a mechanism has been proposed which could account for some features of rapid tumour growth, and attention has been directed to the similarity of conditions in wound healing[2].

The histological appearances and mitotic index have been found to be similar in three different systems which have in common a reduced corticosteroid to growth hormone ratio.

In the first system, Cater, Holmes and Mee[3] showed that injections of pure growth hormone to intact rats caused an increased mitotic rate and synthesis of deoxyribonucleic acid. The mitotic index was approximately double that found in normal rat regenerating liver. The influence of pituitary hormones and cortisone on liver regeneration rate in hypophysectomized rats was then investigated[4]. Cortisone caused complete inhibition of mitosis, which was overcome proportionately as the dose of growth hormone was increased. Growth hormone alone to hypophysectomized rats did not increase the rate of mitosis after partial hepatectomy.

The second system used was the regenerating liver of the adrenalectomized rat in which the rate of mitosis and histological appearances are identical with those of intact rats given growth hormone. A large number of such rats were examined and a study made of the influence of time between adrenalectomy and partial hepatectomy (unpublished work). During the first 48 hr. no inhibiting influence is present and an approximate doubling of normal regeneration rate is seen but after 7 days a return to normal mitotic index occurs.

The third system involved liver regeneration in rats bearing Walker 256 carcinomas. Observations were made of resting liver mitoses, of the mitotic rate 29 hr. after partial hepatectomy, of adrenal weights and of liver glycogen as an indication of corticosteroid output at that time. Two groups were compared, rats bearing large fast-growing tumours and those bearing small slow-growing tumours. It was found that resting liver mitosis rates averaged seven times greater in tumour-bearing rats than in normal rats. Regenerating liver from the fast-growing tumour-bearing rats had a very high mitotic index, not significantly different from that found in adrenalectomized rats. Regenerating liver of slow-growing or arrested tumour-bearing rats had a mitotic index equal to or less than that of normal rat regenerating liver. Liver glycogen in the two groups was significantly different, the larger fast-growing tumour rats tending to have less liver glycogen than liver from a normal rat, while the slow-growing tumour rat deposited about twice the liver glycogen of a normal rat. These glycogen estimations were not regularly consistent but are believed to indicate trends in circulating corticosteroid values.

Taken together, the findings from tumour-bearing rats suggest lower corticosteroid levels in those rats with the fastest-growing tumours. The liver mitosis rates and histological appearances are similar to those found in adrenalectomized rat regenerating liver and the growth hormone-treated rat. These are 'growth hormone like' appearances due in the first two sytems to reduction in the ratio of corticosteroids to growth hormone. In the third system of tumour-bearing rats either a reduction of circulating corticosteroids or some reduction of their effectiveness would account for the similar mitotic index and histological appearances.

Some evidence is being obtained from experiments not yet completed that corticosteroids can be rendered ineffective with regard to their influence on mitosis. It is known from system two mentioned above that removal of corticosteroids from the rat allows an approximate doubling of the normal rate of mitosis in regenerating liver. From experiments of workers[5,6] in the field of ascorbic acid relationship to corticosteroid synthesis and as a direct result of an experiment demonstrating corticosteroid influence on the radiation effect[7], it seemed possible that certain redox chains depending for their integrity on the presence of ascorbic acid were permissive in allowing corticosteroid activity. It seemed that a 'functional adrenalectomy' might be obtained in the guinea pig by deprivation of ascorbic acid so that partial hepatectomy in this animal (unreported work) might reveal a doubling of mitosis-rate over that of normal guinea pig regenerating liver, as has been found in the adrenalectomized rat. The

difficulties in this procedure are being overcome and will form a full report as soon as statistically satisfactory results are obtained. To date 9 normal guinea pigs have survived partial hepatectomy and show a mean regeneration rate of 24 mitoses per 1,000 cells. Mortality among scorbutic guinea pigs is high but of 30 animals 3 have survived with a mean mitotic index of 50. This is a positive result which is unlikely to occur under any other conditions and each individual result should have meaning. To support this, 10 other animals have survived which were believed to have been fully scorbutic but which showed no mitoses in the liver after the same regeneration time. This is consistent with the idea of a low threshold-level of ascorbic acid, and until this is reached, adequate though small amounts of ascorbic acid permit corticosteroid inhibition of mitoses. Very high levels of circulating corticosteroids are known to occur in scorbutic animals[8] and these undoubtedly inhibit all mitoses.

If withdrawal of corticosteroid influence is a likely means of instigation of growth processes, some special mechanism must occur in wound-healing to overcome locally the influence of the raised corticosteroid circulating levels which are inimical to the processes of inflammation and repair. It is suggested that damaged cells may secrete or produce an oxidizing agent in minute amounts which exerts an influence only in the area of damage. Thus neither ascorbic acid nor corticosteroid levels in the circulation need be depleted and may not be found changed on investigation. It is known that following X-irradiation in animals excretion of dehydro-ascorbic and di-ketogulonic acids is increased[9].

These studies have had as an object the mechanism of failure of control of tumour cell growth and it seems likely that tumours act as damaged cells. In addition to their own point of damage which shows itself as, among other things, a failure to respond to circulating corticosteroids[10], it is likely that a spreading influence, at first confined to the immediate cell boundary, but later in an attenuated form perfusing the circulation, renders the host more liable to establishment of metastases and to multiple tumours. Some very recent evidence[11] supporting this concept is of the growth of dormant injected tumour cells in the liver after mere handling of this organ. The fact that an injected tumour-cell population needs to be of a minimum size for survival suggests some ability to influence the host which may not be only of an antigenic nature.

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Effect of Chlorpromazine on Tissue Metabolism *in vitro*

It has been pointed out that central inhibition is connected with an increase of acetylcholine content in brain tissue. On the other hand it has also been shown that some central depressants reduce the oxygen uptake of normal respiring brain tissue *in vitro*[1] or in brain slices which were stimulated electrically[2].

In previous experiments we have seen that chlorpromazine as a tranquillizing drug can also enhance the acetylcholine content in the brain of rats[3]. Further results have confirmed the inhibitory effect of chlorpromazine (even in low doses of 1–10 μgm./ml.) on the rate of oxygen uptake in brain homogenates metabolizing glucose as substrate. However, chlorpromazine did not inhibit uptake of oxygen in the presence of succinate[4].

Studying these relationships we have investigated the direct influence of acetylcholine on the respiratory activity of brain homogenates (albino rats) *in vitro*. The oxygen uptake was followed by the direct Warburg technique (buffered Krebs–Ringer phosphate solution, pure oxygen, 37° C.). The brain tissue was homogenized in the presence of eserine. Results are given in Table 1. Eserine alone did not change the respiration significantly. The lack of some changes after acetylcholine is in agreement with the findings of McIlwain[5] obtained in brain slices of the rat. Similar negative results were observed with the oxygen uptake of hemi-diaphragm slices incubated with acetylcholine.

The inhibitory effect of chlorpromazine on the endogenous respiration (no substrate added) of brain homogenates was reached at relatively high concentration of this substance only. This effect was deepened when ammonium chloride was added simultaneously with chlorpromazine (Table 2).

Contrary to the results of Abood[6] obtained in a mitochondrial preparation of the brain, we could not confirm the inhibitory effect of chlorpromazine on cytochrome oxidase activity in brain homogenates. Enzyme activity was followed by oxidation of 5 m*M* *p*-phenylenediamine during the first hour after addition of chlorpromazine to the brain homogenate. In controls the mean value with standard error of 6 exp. was 143·6 ± 4·7 (μl. oxygen)/100 mgm. wet

Table 1. Oxygen Uptake of Brain Homogenates and Diaphragm Slices after Eserine and Acetylcholine. Medium: Buffered Krebs–Ringer Solution with 0·2 per cent Glucose. Changes in percentage are not Significant

No. of experiment	Tissue	Treatment	μl. O_2/100 mgm. wet w./hr.		Changes (per cent)
			Mean	S.E.	
6	Brain homogenate	Control	112·77	± 1·63	
6		Eserine salicyl. (83 μgm./ml.)	107·32	± 2·93	− 4·85
6		Eserine salicyl. (83 μgm./ml.)	107·32	± 2·93	
		Eserine salicyl. (83 μgm./ml.) + acetylcholine/ml.:			
5		10 μgm.	106·91	± 4·07	− 0·39
5		100 μgm.	110·85	± 4·77	+ 3·28
5		300 μgm.	109·13	± 3·44	+ 1·68
6		833 μgm.	109·29	± 3·29	+ 1·83
9	Hemidiaphragm slices	Eserine salicyl. (83 μgm./ml.)	96·48	± 4·45	
9		Eserine salicyl. (83 μgm./ml.) + acetylcholine (100 μgm./ml.)	99·66	± 5·52	+ 3·3

Table 2. Inhibition of the Endogenous Respiration after Addition of Chlorpromazine and Ammonium Chloride in Brain Homogenates. Medium: Krebs–Ringer Solution (no substrate added). The Inhibition is Significant

No. of experiment	Treatment	μl. O_2/100 mgm. wet w./hr.		Inhibition (per cent)
		Mean	S.E.	
9	Control	82·72	± 1·98	
6	Chlorpromazine (200 μgm./ml.)	62·73	± 3·08	− 24·17
8	NH_4^+ (60 m*M*)	64·74	± 2·87	− 21·8
9	NH_4^+ (60 m*M*) + chlorpromazine (200 μgm./ml.)	51·87	± 3·9	− 37·3

weight (hr.) and after addition of 200 μgm. chlorpromazine/ml. the oxygen uptake was 143·2 ± 3·5.

We have reviewed the specificity of the action of chlorpromazine on cerebral tissue. It did not influence the oxygen uptake of rat's hemi-diaphragm slices metabolizing glucose as substrate. In control animals the mean value with standard deviation of 7 exp. was 95·5 ± 13·4 (μl. oxygen)/100 mgm. wet weight (hr.) and after 10 μgm. chlorpromazine/ml.—94·7 ± 10·3. In 6 exp. after addition of 200 μgm. chlorpromazine/ml. the oxygen uptake showed the value 94·4 ± 11·3.

It can be assumed that chlorpromazine produces an impairment in energy metabolism of the brain tissue such as depletion of oxygen uptake and inhibition of oxidative phosphorylation[7]. On the other hand chlorpromazine exerts no significant changes in cholinesterase activity of the brain as reported by Decsi[8] and it does not affect the synthesis of acetylcholine studied in brain homogenates of the rat[9]. These negative results favour the view that the increase of acetylcholine content following chlorpromazine is due to the depression of central activity without direct relation of chlorpromazine to the acetylcholine metabolism of the brain.

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Action of Thyroxine on the Rate of Oxygen Consumption of the Lung-Fish (*Protopterus*)

The part that endocrines play in the control of metabolic rate of fishes has been investigated by numerous authors. The results concerning the action of thyroxine are contradictory[1–5]. This is mainly due to two essential factors: (1) feeding; (2) activity during measurements[6]. The current investigation was initiated to determine the effect of thyroxine upon the rate of oxygen consumption of *Protopterus*. These fish do not present the above-mentioned difficulties.

Two young individuals were captured from Tienaba (in the vicinity of Dakar), in February (during the aquatic phase), and were maintained in water until the beginning of the experiments in April. Each fish served as its own control, and thus individual variations were eliminated. The first presented external gills, the second only vestigial ones. The total wet weight of the fish was 27·5 and 46 gm. respectively. (The initial weight of the animals before the experiments was the only one taken into consideration.)

The rate of oxygen consumption was measured by means of Haldane's blood gas volumeter, slightly modified for this purpose. The reaction bulb was replaced by a respiratory chamber, inside which was introduced a small quantity of water, the animal and a small tube containing a carbon dioxide absorbent. This apparatus permitted us to measure the volume of oxygen absorbed by the animal to the nearest 0·01 c.c.

The normal rate of oxygen consumption of each individual was followed during the three days preceding the thyroxine injection and several readings taken. Each fish received a single intra-muscular injection of approximately 0·5 c.c. of thyroxine (Roche), in the dorsal muscles.

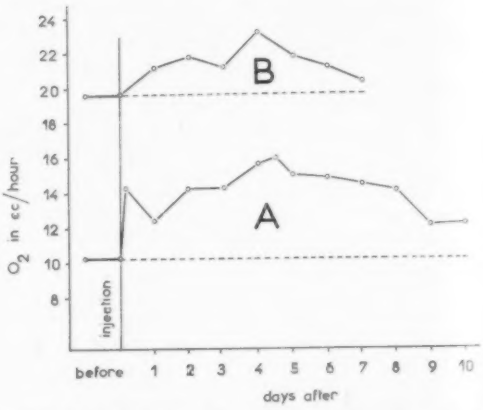

Fig. 1. Action of thyroxine on the rate of oxygen consumption of *Protopterus*

In both cases there was a remarkable increase in the rate of oxygen consumption, one day after injection (Fig. 1). The respiratory rate was maximal about the fourth day, then it dropped gradually during the following days in order to reach approximately its initial level.

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Presence of 5-Hydroxytryptamine after Raunescine in Blood Plasma *in vivo*

5-HYDROXYTRYPTAMINE is localized in the platelets of the blood, minimal amounts being found in platelet-free plasma[1–3]. *Rauwolfia* alkaloids are known to lower the content of 5-hydroxytryptamine in various tissues including blood both *in vivo* and *in vitro*[4,5]. Whether 5-hydroxytryptamine is released by *Rauwolfia* alkaloids from the cellular binding sites as such to the cytoplasma is still not clear. It has been stated that the incubation of rabbit platelet suspensions with reserpine in an atmosphere of nitrogen increases the amount of 5-hydroxytryptamine in plasma[5].

We have investigated whether 'free' 5-hydroxytryptamine can be found in plasma after a *Rauwolfia* alkaloid. As in the earlier experiments[6], which originally directed our attention to this problem, we have used raunescine, which is an alkaloid chemically and pharmacologically closely related to reserpine[7].

3 ml. of blood was collected by using a polyethylene cannula from the abdominal aorta of male rats (160–180 gm.) anæsthetized with ether. Each time one of the rats served as a control and the three others that received 5 mgm./kgm. of raunescine intraperitoneally were bled 1, 3 and 6 hr. after the injection. The blood was mixed with 1/10 vol. of 1 per cent disodium ethylenediamine tetraacetate and 30–50 int. units of heparin/ml. After separation of plasma and platelets[8] the platelet-poor plasma was extracted by 19 vol. of acetone[9] and the packed platelets, after suspension in acid saline, by 10 vol. of acetone. The samples were kept at around 0° C. during the whole pre-extraction period. The biological assay of 5-hydroxytryptamine was made by using the rat stomach preparation[10]. For collecting the blood from the rabbits a catheter was inserted into a cervical vein under light ether anæsthesia, from which the animals were allowed to recover before the withdrawal of blood. In half of the experiments the animals were pre-treated with 100 mgm. (as base)/kgm. of iproniazid phosphate subcutaneously about 16 hr. before raunescine.

The results from rats are presented in Fig. 1. There was a gradual fall in the platelet 5-hydroxytryptamine, and after 6 hr. about 10 per cent was left in the non-pretreated rats and 14 per cent in those receiving iproniazid. Platelets were counted in some tests and they had about 1 μgm. of 5-hydroxytryptamine/10^9 cells on both series. Raunescine did not change the number of them. In the plasma there was a rise in both groups when analysed 1 hr. after raunescine administration. The increase was more than 10-fold in the pre-treated rats, being less marked in the others. The return to normal was also faster in the animals that received raunescine only.

Essentially similar but less consistent results were obtained by using rabbits. The increase of plasma 5-hydroxytryptamine was not as marked as in rats and it was negligible without iproniazid pre-treatment.

The 5-hydroxytryptamine in plasma probably comes from the platelets; but the possible release from other tissues cannot be excluded. The highest amounts of amine in the plasma were found at the time of the most marked release from the platelets. It has been reported that human serum oxidizes 5-hydroxytryptamine[11]. This activity could be inhibited by iproniazid, ceruloplasmin being suspected as the enzyme involved. Therefore the effect

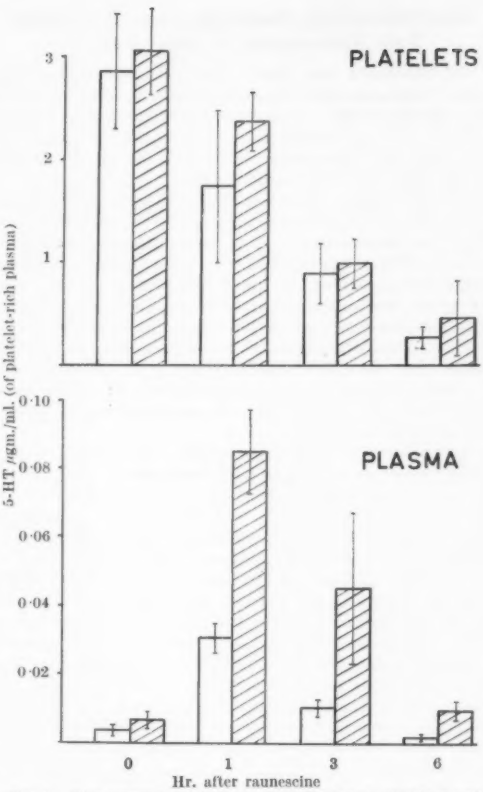

Fig. 1. The amounts of 5-hydroxytryptamine in platelets and platelet-free plasma of rats after raunescine. Each column represents the results from four experiments. Open columns, no pre-treatment; shaded columns, iproniazid pre-treatment; vertical lines, standard deviation

of iproniazid in our experiments may not be due to the inhibition of monoamine oxidase. It is interesting to note that the symptoms produced by raunescine become fully developed at a time when the plasma 5-hydroxytryptamine has returned to normal.

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Adrenocortical Function after Prolonged Salt Restriction in Hypertension

It has been suggested that the high salt intake current in most modern societies is beneficial by virtue of a presumptive stimulating action on the adrenal cortex[1]; it also has been reported[2] that *in vitro* production of corticosterone by the isolated adrenals of sodium-depleted rats declined progressively as a function of time, from which it has been inferred that limitation of salt intake might impair adrenocortical function resulting in an organism less responsive to stress. By contrast, there is considerable evidence implicating sodium in the hypertensive process both of man and animals[3]; furthermore, some results suggest that a high salt intake may be a causal factor in the etiology of human 'essential' hypertension—the second commonest cause of heart disease in Western society[4]. According to the latter view, a high salt diet would not be beneficial and might be frankly harmful.

It has been clearly established by many studies that under normal conditions metabolic balance can be maintained easily on daily salt intakes of 0·5–1·0 gm.—or approximately one-twentieth the amount commonly eaten by the average Western adult[4c]. A number of adrenocortical function studies made on man after short-term salt restriction have shown normal corticoid production with variable evidence of increased aldosterone production (for example, ref. 5). The long-term effects of salt restriction on adrenocortical function have not been studied but have assumed increasing importance with the widespread use of low salt diets in medicine: it is this aspect with which we are concerned here, brief mention of which was made in an earlier communication[4b]. A unique opportunity to make such observations was provided by 3 ambulatory subjects maintained on accurately measured diets, which contained only 100–150 mgm. of sodium (250–375 mgm. as sodium chloride) daily, for periods of from 1 to almost 5 years. We are aware of no natural diet which is so restricted in sodium as that used in the present work, and no suggestion is intended that comparable levels are necessary or advisable for the population generally: the present regimen for these patients was a deliberately severe test of the adaptive capacities of the adult organism carried out under careful supervision. The subjects included a 69-year old white widow and 2 white bachelors of 49 and 51 respectively (ages on admission). All had classical uncomplicated essential hypertension; they were ambulatory and went for long walks on the laboratory grounds ordinarily at least once daily. 24-hr. urine collections were made continuously and at no time was there evidence of extra salt intake. Numerous studies carried out on these individuals have been omitted unless clearly relevant to the current discussion. It is pertinent to note that on admission the 49-year old male was found to have an eosinophilia of 4–15 per cent which was not explained and which persisted in the hospital.

The observations reported here, unless otherwise indicated, were made after 58, 32 and 20 months on low salt regimen by the 69-year old woman, and the 49- and 51-year old men, respectively. Physically, in terms of disease entities or syndromes known to be related to the adrenal cortex, these subjects presented no evidence of either hypo- or hyper-corticism. All three had some personality peculiarities on admission which did not change with salt restriction: they appeared normal and acted normally and reported that they felt normal. The following laboratory studies, some of which may be abnormal with various states of adrenocortical dysfunction, were normal and essentially unchanged from the results obtained prior to salt restriction: plasma protein and specific gravity; hæmoglobin, hæmatocrit, white blood count and differential; urea clearance and blood urea nitrogen; plasma sodium, potassium, chloride and carbon dioxide; total exchangeable sodium as measured with sodium-24. Blood sugars remained normal both after eating and after a 24-hr. fast; intravenous glucose tolerance tests showed normal curves. The response to intravenous adrenocorticotrophic hormone[6] is summarized in Table 1. A normal fall in total circulating eosinophils occurred following the infusion of adrenocorticotrophic hormone, to 10 per

Table 1. 48-hr. Adrenocorticotrophic Hormone Test for Adrenocortical Reserve Function

Day	♀ 69				♂ 49				♂ 51			
	1 (Control)	2 (Control)	3 (Test)	4 (Test)	1 (Control)	2 (Control)	3 (Test)	4 (Test)	1 (Control)	2 (Control)	3 (Test)	4 (Test)
Eosinophils—mm.³ (normal: 100–250)	63	69	0	6	460	413	25	38	181	166	19	3
Urinary: 17-ketosteroids, mgm./24 hr. (normal: ♀ 5–15 ♂ 10–20)	9·7	10·0	9·0	15·1	10·0	8·2	14·5	28·9	13·0	14·5	21·1	23·8
Urinary: 17-hydroxy-corticoids, mgm./24 hr. (normal: ♀ + ♂ 4–11)	6·8	7·1	24·1	33·5	7·6	7·2	35·3	48·9	9·0	7·1	37·6	49·9
Months off sodium chloride	55				13				12			

Adrenocortical response to 25 units of adrenocorticotrophic hormone infused intravenously on both days 3 and 4.

Table 2. Urinary Steroid Excretion

	Normal range (per day)	♀ 69 Day 1	♀ 69 Day 2	♂ 49 Day 1	♂ 49 Day 2	♂ 51 Day 1	♂ 51 Day 2
Aldosterone	1–10 μgm.	Lost—laboratory accident	12	4	2	30	39
Cortisone	10–114 μgm.	175	163	10	13	62	64
Hydrocortisone	34–262 μgm.	309	412	(not done)		92	113
Months off sodium chloride		56		19		19	

cent or less of control values. The urinary excretion of both 17-ketosteroids and 17-hydroxycorticoids during the control days was within normal range and the subsequent response to the infusion of adrenocorticotrophic hormone indicated a normal adrenocortical reserve. Maintenance of sodium chloride balance during this period of severe restriction is *a priori* evidence of adequate production of salt-retaining hormones although no necessary indication of corticoid production. The daily urinary excretion of aldosterone, cortisone, and hydrocortisone[7] is summarized in Table 2. The significance of the slightly increased excretion of cortisone and hydrocortisone by the woman is doubtful in view of the wide range of normal, and in association with the normal values shown in Table 1 as well as the absence of other biochemical or clinical evidence of increased corticoid production. The aldosterone excretion for the 51-year old male appears elevated to levels reported for some studies[5] after short periods of salt restriction: the other two patients did not show this effect. From the results in Tables 1 and 2 we have concluded that prolonged salt restriction is associated with normal adrenocortical steroid production. We are aware that urinary excretion need not necessarily reflect adrenocortical production of steroids, and these results must be interpreted with this restriction. It is proper to ask whether the results reported here would hold in non-hypertensive individuals. We have subjected non-hypertensive persons to equal salt limitation for periods up to 3 months with similar findings, so it is unlikely that this capacity is peculiar to the individual with hypertension. Furthermore, during the past 12 years in an experience with more than 100 other individuals studied on this same regimen for periods as long as 1 year we have never found evidence of adrenocortical dysfunction.

From these studies, we cannot refute the suggestion that a high salt diet is beneficial but the evidence presented suggests that a prolonged and drastic low salt diet is compatible with normal adrenocortical function.

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Effect of Age on the Heparin Content of Rat Skin

IN 1955, Shetlar and Masters[1] demonstrated a change with age in the composition of acid mucopolysaccharides of human cartilage. They found that both uronic acid and hexosamine decreased with age but the decrease in hexosamine was less striking, indicating a relative increase in some hexosamine-containing polysaccharide. Further evidence for a shift in the relative proportions of acid mucopolysaccharides in ageing cartilage was provided by Kuhn and Leppelmann[2], who reported a decrease in the ratio of galactosamine to glucosamine. Recently, Kaplan and Meyer[3] observed that the content of chondroitinsulphuric acid in human cartilage decreased and that of keratosulphate increased with age. On the basis of an increasing glucosamine to galactosamine ratio, Hallén[4] concluded that the ratio of keratosulphate to chondroitinsulphuric acid in human nucleus pulposus increased with age. A difference in the distribution of mucopolysaccharides between embryonic and mature pig skin was reported by Loewi and Meyer[5]. They found that hyaluronic acid comprised 78 per cent of the total skin mucopolysaccharides in the embryo and only 30 per cent in the adult, whereas chondroitinsulphuric acid-*B* represented 5–12 per cent in embryonic skin and 64 per cent of total mucopolysaccharides in adult skin.

The development of quantitative methods for extracting and separating mucopolysaccharides from tissues has made it possible to investigate the relationship of age and the qualitative and quantitative mucopolysaccharide content of skin. These studies lead to the isolation of heparin in relatively large amount from rat skin. Analysis of skin from rats of various ages demonstrated increased concentrations of all mucopolysaccharides in the very young animal. Most dramatic, however, were the changes in heparin concentration.

Acetone-dried skin from male Sprague–Dawley rats, ranging in age from 21 days to 2·6 years, was extracted by a modification of a method published previously[6]. The mucopolysaccharide mixture was precipitated by cetyl pyridinium chloride in the presence of 'Celite' as described by Korn[7]. Hyaluronic acid, the chondroitinsulphuric acids, and heparin, were extracted by successive washes with 0·3 *M*, 1·2 *M* and 2·1 *M* sodium chloride, respectively.

The results, presented in Table 1, are expressed as μgm. of uronic acid (as determined by the carbazole reaction[8]) per gm. of acetone-dry skin. The concentration of hyaluronic acid and chondroitinsulphuric acid in young rats was found to be approxim-

Table 1. EFFECT OF AGE ON CONCENTRATION OF MUCOPOLYSACCHARIDES IN RAT SKIN

Age (days)	Hyaluronic acid	Chondroitinsulphuric acid	Heparin
	(μgm. uronic acid/gm. dry rat skin)		
21*	979	385	421
23*	894	406	562
44*	1,232	337	388
57*	439	218	100
74*	540	197	92
217*	578	273	111
406	320	140	70
473	450	200	110
561	480	257	68
893	380	135	35
951	538	256	100

* These results were obtained from pools of 8–10 rat skins for each age group. In the older age groups, the results represent values from individual animals.

ately twice that in mature animals. The amount of heparin in young animals was surprisingly large. After 50 days of age the heparin concentration had diminished by 75 per cent. Thereafter, the level of all acid mucopolysaccharides in skin appeared to remain fairly constant.

In order to demonstrate unequivocally that the mucopolysaccharide present in the 'heparin' fraction was indeed heparin, 56 gm. of acetone-dried rat skin were extracted and the mucopolysaccharides separated as above. Approximately 9 mgm. of mucopolysaccharide were isolated from the 'heparin' fraction. The material was subjected to chromatography on 'Dowex 1 × 2', chloride resin, using increasing concentration of sodium chloride for elution. Heparin was removed with 2 *N* sodium chloride. Analyses showed hexosamine, uronic acid (by the carbazole reaction[8]) and ester sulphate to be present in a molar ratio of 1·0 : 1·6 : 2·0, respectively. The amino-sugar, determined by the method of Stoffyn and Jeanloz[9], was glucosamine. The substance had a specific rotation of + 90° and an anticoagulant activity identical to that of a commercial preparation of beef heparin (General Biochemicals, Inc.) with 136 units per mgm.

In view of the large number of mast cells in rat skin, the presence of heparin in this tissue is not surprising. Monkhouse *et al.*[10] reported heparin activity in extracts of rat skin but no chemical analyses were published, and their preparations contained a low order of anticoagulant activity.

Whether the heparin present in skin is entirely intracellular cannot be ascertained from the present data. It is conceivable that some heparin is secreted into the extracellular space during the life-cycle of the mast cell. This conjecture is consistent with a recent observation by R. S. Geiger and A. Dorfman (unpublished results) that granules are extruded from mast cells during culture of a mouse mastocytoma.

The finding of large amounts of heparin in skin and its relative decrease with age has many physiological implications. The role of heparin in lipæmia-clearing and blood coagulation suggests that changes in tissue concentration may influence these phenomena. It is possible that a local decrease in heparin affects deposition of fibrin or lipids.

A more detailed report of the methods and results will be published elsewhere.

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A Possible Function of Planktonic Photophores

A number of unrelated groups of planktonic animals have developed the curious arrangement of upwardly directed eyes combined with downwardly directed photophores; this has been described and discussed by Hardy[1], who has suggested a number of possible explanations.

Could this singular arrangement sometimes constitute a mechanism whereby the members of a vertically migrating species can keep together even when the most important factor, light from the surface, is brusquely interrupted?

Consider a gregarious species, mainly light-controlled, which occupies depths between 600 m. and 800 m. on a fine day, and between 200 m. and 400 m. by night. At midday after a sunny morning the animals at 600 m. will be comparatively well illuminated, but those at 800 m. may be almost at the threshold of perception of daylight. Now suppose that, as must not infrequently happen, the Sun is suddenly obscured by thick cloud for the rest of the day. The highest animals will start to swim up towards the levels of light-intensity that suit them best; the deeper animals, however, cannot at once do so, for they are now suddenly unable to perceive any daylight at all. Their upward-pointing eyes, however, may be able to perceive the diminution, in size and intensity, of the pattern of the photophores of their relatives above them, now rapidly climbing. Since they are gregarious, this situation might urge them in their turn to climb, so as to keep station with their fellows.

In this way, I suggest, it may be possible for a species to undertake light-controlled vertical migrations, even though the light may be acting on many of the individuals only at second hand.

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PLANT PHYSIOLOGY

Interaction of Growth Inhibitor and a Natural Germination Stimulator in the Dormancy of *Fraxinus excelsior* L.

Dormancy of seeds of the genus *Fraxinus* has been variously ascribed to immaturity of the embryo[1], mechanical resistance of the seed coats[2,3], and the presence of growth-inhibiting substances within the pericarp[3] and in the mucilage surrounding the embryo[4]. A recent study of dormancy in the fruits of *F. excelsior* L. has shown that these explanations are inadequate.

When the fruit of *F. excelsior* is shed from the tree, the embryo is small, but morphologically complete. Before germination occurs, the embryo grows by cell division and cell extension from little more than half, to the full length of the seed. This occurs most rapidly in moist storage at warm temperatures (18–20° C.) and is greatly retarded by the presence of the pericarp, which apparently restricts the oxygen

supply to the embryo. Even after the embryo is fully grown, neither the fruits nor the naked seeds germinate unless after-ripened in moist storage at 5° C. for at least 4 months. Excised, unchilled embryos remain dormant indefinitely if precautions are taken to preclude leaching, whereas embryos excised from chilled seeds begin to germinate immediately.

An extensive survey of the changes in growth-promoting and growth-inhibiting substances has been carried out during embryo growth, chilling and germination. Diethyl ether extracts, separated into acid and neutral fractions, and aqueous extracts were concentrated and chromatographed on paper using as a developing solvent 80 parts of *iso*propanol to 20 parts of 0·15 *M* ammonia. The chromatograms were assayed for active substances using the *Avena* coleoptile straight-growth technique, and embryos excised from seeds of *F. excelsior*.

Ether extracts of ungerminated seeds contain no growth-inhibiting substances, but a growth-promoting substance, tentatively identified as indoleacetonitrile by means of its R_F value in various solvents and colour reactions, is present in ether extracts of fresh seeds and gradually disappears during growth of the embryo. Aqueous extracts of dry seeds contain no growth inhibitors, but on imbibing the seeds with water an active growth-inhibiting zone R_F value 0·6–0·8 appears in the chromatograms of extracts of endosperms and embryos imbibed 24 hr., and increases in activity during the following 48 hr. This inhibitor not only prevents the growth of *Avena* coleoptiles, cress roots and the germination of lettuce seeds, but also inhibits radicle growth of excised embryos of *F. excelsior*.

Although the seeds germinate after chilling, no reduction in activity of the inhibitor in either the endosperms or the embryos could be detected during or after the period of chilling. After the initial stages of germination, however, a very slight reduction in activity was detected, accompanied by the appearance of an ether-soluble inhibitor with similar properties to the β-inhibitor of Kefford[5].

Excised, unchilled embryos germinate rapidly if leached in distilled water for 48 hr., whereas embryos excised from leached intact seeds remain dormant.

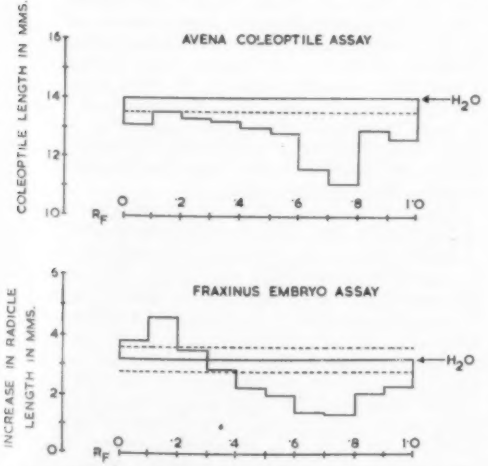

Fig. 1. Aqueous extract of embryos of *F. excelsior*. Chromatogram halved longitudinally, one half assayed with *Avena* coleoptile test, other half assayed with leached *F. excelsior* embryos

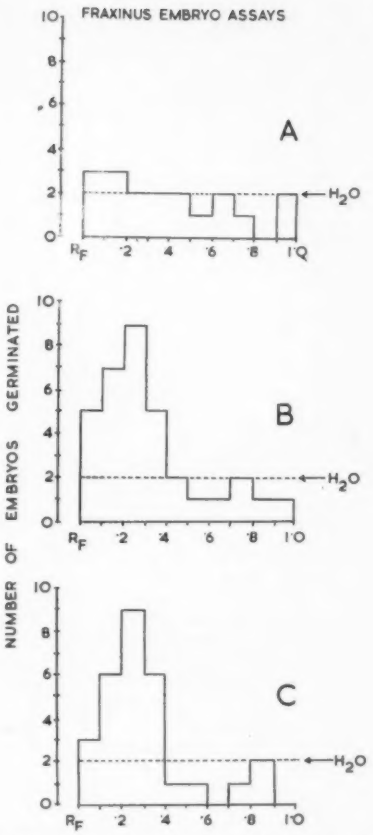

Fig. 2. Effect of aqueous embryo extracts on germination of unchilled embryos. *A*, Extract of unchilled embryos; *B*, extract of chilled, non-germinating embryos; *C*, extract of chilled, germinating embryos

A very slight reduction in the level of inhibitor was detected in leached embryos, but not in the embryos excised from leached intact seeds. If leached (non-dormant) embryos are placed on filter paper moistened with inhibitor solution, very little growth ensues. This was used as the basis for biological assays in which ten leached excised embryos were placed in the eluates from sections of chromatograms in place of the ten coleoptile sections normally used. It was shown that the fraction which prevented embryo growth was identical with the inhibitor of coleoptile growth (Fig. 1), and was active at all stages of after-ripening and germination of the seed.

Thiourea has the property of counteracting the effect of the inhibitor on the germination of lettuce seeds. Excised unleached (dormant) embryos of *F. excelsior* germinate readily if placed on filter paper moistened with 0·5 per cent thiourea solution, but no reduction in the level of inhibitor can be detected within the embryos.

As chilled embryos are able to germinate without leaching or thiourea treatment, and the process of chilling does not decrease the inhibitor content of endosperm or embryos, it was decided to investigate the possibility of the production of a germination-promoting substance. As no such substance could be demonstrated using the coleoptile straight-growth

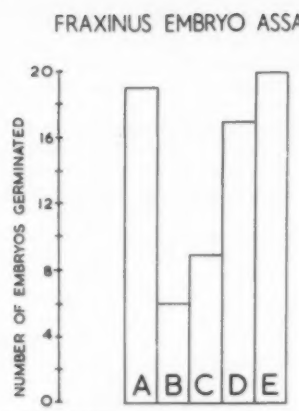

Fig. 3. Interaction between inhibitor and germination promoter. Leached, unchilled embryos on filter paper moistened as follows: *A*, water only; *B*, inhibitor only; *C*, inhibitor + eluate from zone R_F 0·15–0·35 of extract of unchilled embryos; *D*, inhibitor + 0·5 per cent thiourea; *E*, inhibitor + eluate from zone R_F 0·15–0·35 of chilled embryos

Note.—Embryos from *A* produced stunted seedlings, those from *D* would not grow further, those from *E* produced normal seedlings

technique, unleached (dormant) embryos of *F. excelsior* excised from unchilled seeds were used in place of the coleoptile sections in biological assays. It was found that the zone R_F 0·15–0·35 in chromatograms of aqueous extracts of chilled, and of chilled germinating embryos, stimulated the germination of the dormant assay embryos within three days. This zone of stimulation was shown not to be present in extracts of unchilled embryos (Fig. 2), nor in the endosperms of seeds at any stage of after-ripening or germination.

That this germination-stimulating substance has the property of counteracting the effect of the inhibitor was demonstrated by the following experiment. The inhibitor-containing zone of a chromatogram (R_F 0·6–0·8) was cut into four strips and the strips moistened separately in Petri dishes with: (*a*) distilled water as a control; (*b*) eluate from zone R_F 0·15–0·35 from a chromatogram of extract of 10 unchilled embryos; (*c*) 0·5 per cent thiourea solution; (*d*) eluate from zone R_F 0·15–0·35 from a chromatogram of extract of 10 chilled embryos. A further control of filter paper moistened with distilled water (that is, no inhibitor) was prepared. Twenty unchilled, leached (non-dormant) embryos were placed in each of the above five dishes. It was found that there was little growth in the dishes containing either inhibitor alone, or inhibitor plus eluate from unchilled embryos, whereas in the dishes containing inhibitor plus eluate from chilled seeds, inhibitor plus thiourea, and distilled water alone, full germination took place within four days (Fig. 3).

The subsequent growth of variously treated embryos was investigated. Seedlings grown from excised chilled embryos, and excised unchilled embryos treated with the germination stimulator extracted from chilled embryos, were apparently normal when compared with those produced from normally germinated after-ripened seeds. Seedlings produced from unchilled embryos, germinated after leaching in water, were stunted, and embryos treated with thiourea did not produce seedlings, even after washing to remove excess thiourea.

Tests in which embryos in various stages were treated with synthetic gibberellic acid have shown that although the dormant embryos can be stimulated to germinate slowly by treatment with this substance, nevertheless the stunting of seedlings grown from unchilled embryos is not removed by gibberellic acid. It would seem, therefore, that the germination promoter is not gibberellic acid, a conclusion which is supported by the fact that small amounts of tissue may be used for extraction, in contrast with the large amounts used for the demonstration of natural gibberellins.

The fact that the inhibitors and germination stimulator may be detected by the embryos in which they occur naturally is good evidence that the results obtained *in vitro* reflect actual changes within the after-ripening seed. The evidence obtained suggests that the deep dormancy of seeds of *F. excelsior* is imposed by the presence of growth inhibitors in both endosperm and embryo. During chilling, this dormancy is overcome by the production of a germination-stimulating substance in the tissues of the embryo.

A detailed account of the experimental work will be published elsewhere.

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Effects of Gibberellic Acid on Excised Pea Roots

In the course of an investigation of inter-varietal differences in the growth of excised roots of field peas (*Pisum sativum* L. var. *arvense*), some effects of gibberellic acid have been observed which seem worthy of brief record. Seeds were surface sterilized with 0·1 per cent mercuric chloride and germinated for two days. Root tips (5 mm.) were excised and grown in the dark at 25° C. in Petri dishes containing the inorganic medium of Bonner and Addicott[1] with 4 per cent sucrose, vitamin B_1 (0·1 mgm./l.) and nicotinic acid (0·5 mgm./l.). Ten tips were employed in each treatment, two being grown in each dish. After seven days the roots were measured and the apical 10 mm. of each was transferred to fresh medium for a further week.

Roots of the variety Minerva Maple grew extremely well (Table 1) and, so far as is known, no other variety of either field pea or garden pea has been found to grow as rapidly under similar conditions. By comparison, the growth of the variety Winter Maple was poor and it decreased markedly in the second passage. Gibberellic acid did not influence the growth of Minerva and in this respect this variety behaved in a similar way to the roots of intact cress seedlings[2]. With Winter Maple on the other hand, 0·5 and 25 mgm./l. gibberellic acid caused significant though small increases in growth in the first passage. The lower concentration stimulated growth again in the second passage but the higher concentration gave no stimulation. When seedlings of Winter Maple, which

Table 1. Effects of Gibberellic Acid on Excised Roots of Minerva Maple and Winter Maple

Variety	Concentration G.A. (mgm./l.)	Length of root (mm.)	
		Passage 1	Passage 2
Minerva Maple	0	77·6 ± 7·0*	87·5 ± 10·0*
	0·5	71·8 ± 4·0	90·6 ± 7·7
	25·0	89·0 ± 8·7	83·5 ± 9·5
Winter Maple	0	41·9 ± 1·8	15·7 ± 2·1
	0·5	48·8 ± 2·5	25·8 ± 1·1
	25·0	46·7 ± 1·7	14·7 ± 2·1

G.A., Gibberellic acid. * Standard error.

is not a dwarf variety, were grown in an inorganic medium the roots grew much more rapidly than the excised roots in sterile culture. The above results may indicate that one of the factors limiting the growth of the excised roots of Winter Maple is a gibberellic acid-like substance.

The most striking effect of gibberellic acid on the excised roots, however, was on the development of root hairs. In most plants, root hairs are formed much more readily in moist air than in water[1]. In excised pea roots some hairs form on the tissue of the inoculum but the major part of the root is normally hairless. However, in the presence of gibberellic acid root hairs continued to develop in an acropetal manner, the youngest occurring on the tissue which had just completed extension growth. The covering of hairs was considerably denser at 25 mgm./l. than at 0·5 mgm./l. In Minerva the hairs were quite normal in appearance. In Winter Maple the hairs were shorter than in Minerva and at the higher concentration of gibberellic acid they tended to swell at the tip, the region of the hair which is generally believed to exhibit the greatest plasticity[2].

That gibberellic acid stimulates the normal development of hairs on excised roots raises the possibility that substances of this type may participate in the formation of these structures in intact plants.

The seeds employed in this work were kindly supplied by the Plant Breeding Institute, Cambridge.

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Reversal of Genetic Dwarfism in *Tephrosia vogelii* by Gibberellin

In conjunction with a breeding programme designed to produce plants of *Tephrosia vogelii* Hook f. with a higher rotenone content, multiple crosses were made between two lines of similar morphology, but of separate geographical origin. Nine dwarf plants were obtained as segregants in an F_2 population of 103 individuals. These dwarfs were characterized by greatly reduced internodes, hypocotyls, petioles, and rachises. Leaflets were also much smaller, and leaf colour was a darker green than normal. The number of leaves per plant, the number of leaflets on corresponding leaves, and the size and colour of the cotyledons were unaffected.

The potassium salt of gibberellic acid in an aqueous solution of 100 p.p.m. was applied in the form of a light spray to three of the dwarf plants. Three additional dwarf plants were sprayed with indoleacetic acid at 100 p.p.m., and the remaining three were sprayed with water as a control. The initial applications were made approximately one month after germination, and an additional spraying was given one week later. After responses to gibberellin were noted, two of the plants in this treatment were withheld from further applications, while one plant continued receiving weekly treatments of gibberellin.

Elongation of the petioles, rachises and leaflets was observed one week after the initial application of gibberellin. Leaves and internodes which had elongated prior to treatment were unaffected. Two weeks after the initial treatment with gibberellin, the three plants showed elongated internodes and leaves which were identical in appearance to those of normal seedlings. Normal growth continued in the plant which received additional periodical treatments of gibberellin. In those plants which were withdrawn from the gibberellin treatment after two applications, the dwarf condition reappeared gradually, and one month after the last treatment the plant was indistinguishable from the dwarf controls except for the elongated stem produced during the period of gibberellin treatment. These results indicate that the dwarf segregants of *T. vogelii* are completely dependent on periodic applications of gibberellin for normal growth.

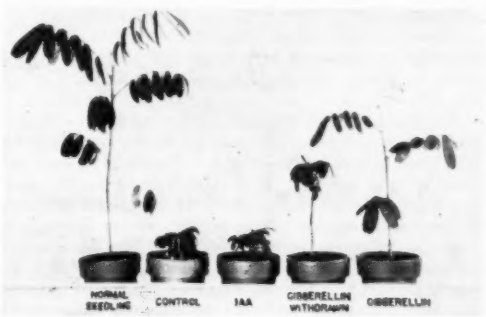

Fig. 1. Seedlings of *Tephrosia vogelii* Hook f. showing a normal F_2 seedling, an untreated dwarf segregant, a dwarf treated with indoleacetic acid, a dwarf treated and then withdrawn from gibberellin, and a dwarf on continued gibberellin treatment

There was no growth response in plants treated with indoleacetic acid when compared to the controls. Fig. 1 shows (from left to right) a normal seedling of *T. vogelii*, an untreated dwarf segregant, a dwarf treated with indoleacetic acid, a dwarf treated with gibberellin for two weeks and then withdrawn, and a dwarf which has continued to receive periodical applications of gibberellin. A similar reversal of genetic dwarfism in other legumes[1] and in maize[2] has been reported.

We are indebted to Merck Sharp and Dome International, Rahway, New Jersey, for supplying the gibberellin used in this study.

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BIOLOGY

Water Hyacinth : a Curse or a Crop?

An organism often multiplies explosively when carried to a new environment. Rabbits in Australia are a familiar example, and now we have water hyacinth (*Eichornia crassipes*) in south-east Asia, the Nile, and the Congo[1–3]. In time, enemies of the invader will probably evolve in the new environment, or be introduced into it, and restore a balance, but that may take many years. In the meantime, there is disruption of old patterns of life and effort is therefore put into attempts to eradicate the invader and restore the *status quo*. This is obviously wise, but it is by no means certain that eradication will be successful, so that it may be prudent to try to find how best to live in the new circumstances. The invader may often be useful.

The main disadvantages of working with a weedy crop, or with wild vegetation, are that there is competition and products are harvested with widely different properties and potentialities. They would be useful separate but not mixed. Water hyacinth produces a pure stand. Acres of water that hitherto carried little plant-life now carry a rich growth. This makes the plant resemble a crop rather than a weed and raises a fundamental issue. Hitherto, agricultural research has been based on the idea that we know what crops we want and then grow them in increasing abundance in increasingly exacting environments. Biochemical engineering introduces the opposite outlook : it strives to find what an area will produce most abundantly and then looks for a use for it[4].

A floating plant is ideally adapted for mechanized harvesting but possible uses for water hyacinth have not been intensively sought. Cattle will eat it, as Davies[2] pointed out, and Hora[5] describes its use as pig food in south-east Asia. It has been fermented to give methane[6] and compost[7]. When harvested it contains 95 per cent of water. This is a disadvantage for many uses, but is a great advantage if it should prove possible to use the leaf as a source of extracted protein, because general experience suggests (cf. ref. 8) that protein extraction is easier from wet than from dry leaves. Leaf protein extraction is now being studied in several places with many different types of leaf, but the basic principle of extraction is always the same and is very simple[9] ; the fresh leaf is pulped and the juice, neutralized if necessary, is separated. It carries with it much or most of the protein, and this is coagulated and filtered off as a dark green cake which generally has little flavour after washing with water. It can be used as it stands in various dishes that are reasonably palatable[10], or a stable protein can be made by extracting the chlorophyll and lipids with a solvent. So many species have now given satisfactory yields of protein that there is good reason to expect that it could be extracted from water hyacinth. But this has not, apparently, yet been demonstrated.

This communication is a plea for some re-orientation of thinking in areas suffering from, or threatened with, water hyacinth. There is a case for directing some research towards finding uses for the pest instead of concentrating all efforts on its eradication. The practicability of those mentioned here has not been established and difficulties may arise with some of them. But the quantity of water hyacinth is now so large, and so much effort is already being put into dragging it from waterways, that it would seem reasonable to set up a unit to study all the possibilities.

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Chick Feeding in the Wandering Albatross *Diomedea exulans* Linnæus

It has long been known that the young of the wandering albatross (*Diomedea exulans*) do not fly until eight or nine months after hatching, and the problem of how this long fledgeling period fits into the breeding cycle of the adults has caused some speculation among naturalists acquainted with the species[1–6]. Excellent descriptions are available of the birds at sea and on the breeding grounds, but it is only recently that detailed study within a large population has been undertaken[7].

As early as 1929 it was suggested that individuals of the species may not breed each consecutive season because of the necessity of feeding the young throughout their first winter[1]. Such a phenomenon was held by Murphy[2] to be highly improbable. It was maintained that the only hypothesis consistent with the few other available winter observations at breeding stations[6] was that the chicks, after being fed for the first three or four months, were deserted by their parents, who departed to sea to prepare for the following breeding season. The nestlings, it was stated, went for the last four months, throughout their first winter, without feeding but surviving off stored body reserves of visceral and subcutaneous fat until fully fledged and able to leave the nesting grounds, shortly after the adults had returned again to breed.

Studies of the related royal albatross (*Diomedea epomophora*) have since demonstrated a bi-annual breeding cycle in which the young are fed throughout the fledgeling period of 236 days[4]. Since Matthews's original description[1], winter feeding in the wandering albatross has been noticed at three other stations. In the Antipodes Islands eight-month-old chicks were receiving meals[3] and at Kerguelen feeding has been seen during August and September[6]. On Gough Island, adults were observed with eight-month-old chicks some time before the return of breeding birds[10].

Recent workers at South Georgia[7] spent five months in the field during October 1958–March 1959, most of it on Bird Island 54° 00′ S. ; 38° 02′ W., the densest breeding ground of the wandering albatross in the world.

The albatross returns to South Georgia in mid-November ; but before the return of the main body of breeding birds single adults were observed paying brief visits to nestlings of not more than 10 min. duration. Banding operations began at Bird Island on November 26 and during the following month 656 nestlings were banded. In the course of this

work, nestlings being handled frequently regurgitated large fresh meals and the sight of adults feeding young was fairly common. There is no direct evidence that these adults were in fact feeding their own young; but nestlings were often seen begging from adults which refused them. Conversely, there is no indication in the royal albatross that adults feed random chicks[4].

In adult wandering albatrosses there is a significant difference in body-weight between the sexes, but at the time of departure the few young examined were not necessarily lighter or heavier than the adults. The range of body-weights in twelve juveniles a few days before leaving varied between 5·6 and 11·3 kgm. while that of 105 adults of both sexes was 6·35 to 9·15 kgm.

Table 1. WEIGHTS OF ADULT WANDERING ALBATROSSES, BIRD I

Sex	No.	Mean (kgm.)	Standard deviation (kgm.)	Standard error (kgm.)	Range (kgm.)
Male	52	9·11	0·60	0·083	8·20–9·15
Female	53	7·27	0·52	0·071	6·35–7·30

The lightest of the juveniles had been losing weight at the rate of 95 gm./day during the preceding twelve days while the mean daily loss of the heaviest over eighteen days was 200 gm./day (Fig. 1). The combined mean daily loss in four juveniles during the three weeks preceding departure was 162·5 gm./day (No. 64, *S.D.* 3·30, *S.E.* 0·412, range 1–650 gm.).

Thus a young albatross leaving the breeding grounds in December with a flight weight of 9 kgm. must, if it had fasted the whole winter, have weighed over 23 kgm. the previous July—more than twice the body-weight of an adult male. Rankin[3], calculating from daily weight increments of four chicks in the month following hatching, estimated that the weights at the end of June would be between 30 lb. 12 oz. and 25 lb. 13 oz. (13·9–11·8 kgm.). In comparison with adult weights these appear to be reasonable estimates, but they are strictly comparable with those of fully fledged juveniles preparing to depart in December. Thus they are no evidence for the hypothesis that they are put forward to support; rather the reverse, for young albatrosses of such peak weights would not have survived a winter without fairly regular feeding.

The young of most of the procellariiformes reach peak weights in excess of the parent body-weights.

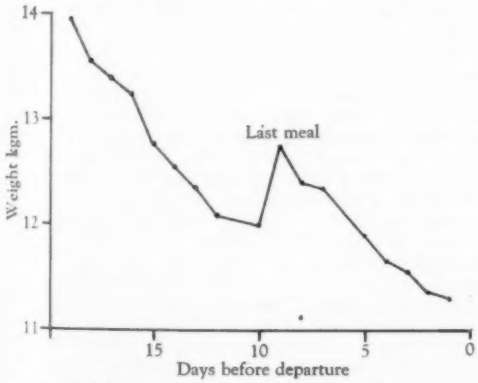

Fig. 1. Weight of a young wandering albatross during its last weeks at the nest

In some species there may be desertion by adults but recent studies have demonstrated that it is more usual for the young to lose surplus weight during the latter part of the fledgeling period while they are still being fed[4,11–15]; although this feeding is less frequent than during the earlier part of the chick period.

While nothing can yet be deduced of the feeding regime of the wandering albatross, the evidence now available clearly indicates that the young are fed throughout the fledgeling period. The 'starvation' hypothesis is therefore untenable and it must be held that the wandering albatross, like the royal albatross, breeds only once every two years.

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Hydrophilic Gels for Biological Use

PLASTICS to-day enjoy wide use in many fields, and it is natural that the possibility of their employment in permanent contact with living tissues has been seriously considered. A study of the literature shows that almost all known plastics have been proposed for this purpose at various times. The question of structural similarity with the tissue has not, however, been emphasized although physiologically unfavourable effects were observed in most cases of application of normal type plastics.

The extensive application of plastics for alloplastic and prosthetic uses requires solution of the problem of compatibility of the material with the live tissue. It is indisputable that non-biological material represents a foreign body having contact surfaces showing large deviations from normal physiological behaviour.

The result of poor compatibility is either an immediate severe local irritation or a tumorous growth which sometimes appears only after a longer interval. The cause of these phenomena, as has been ascertained, is to be found among other factors in the mechanical irritation or in the long-term slow diffusion of low molecular irritant substances from the plastic itself, so that every plastic must be judged from this point of view. All high polymers used so far in medicine are impermeable to metabolites. The majority of them are hard solids so that they can cause mechanical irritation. Further, one must expect a certain slow leaching of irritating substances from them. Polyvinyl alcohol is an

exception; but it has the major disadvantage that it is absorbed.

The demands to be placed on a suitable plastic after consideration of the above-mentioned effects are thus: (1) a structure permitting the desired water content; (2) inertness to normal biological processes (including resistance to the degradation of the polymer and to reactions unfavourable to the organism); (3) permeability for metabolites.

Materials with these properties must have hydrophilic groups. Further, they must have a three-dimensional structure with at least enough cross-linkages to prevent absorption.

Of a large number of plastics we found co-polymers of glycolmonomethacrylate with several tenths per cent of glycoldimethacrylate to be the most suitable. They hydrolyse only with difficulty, even under severe conditions, and are indifferent to biological materials. A further advantage is that their mechanical properties and water content can be adjusted to meet requirements. They withstand heat sterilization without damage.

Hydrophilic materials of a marked polyelectrolyte character (with basic groups, or with carboxyl groups) have unfavourable effects on the organism, swell excessively and their chemical stability does not attain the stability of glycol esters.

Gels based on glycolmethacrylates are also noteworthy for their transparency (in the case of polyglycolmonomethacrylate with a water content less than 30 per cent and with the higher glycols at all concentrations). The water content, and thus the shape of the object, is, after attaining equilibrium at the given temperature, constant and depends on the structure and number of cross-linkages. The appropriate monomers can be prepared by the alcoholic re-esterification of methylmethacrylate with glycol followed by selective extraction of the di- and mono-esters from the reaction mixture. The desired shapes are prepared by polymerization of aqueous solution in suitable moulds. Rubber-like and soft gels can be stiffened by polyamide fibres either in the form of flock or threads. Before use it is necessary thoroughly to leach out the shapes.

The formation of a porous structure by the rapid polymerization of glycolmonomethacrylate is of great interest. With a water content greater than corresponds to the equilibrium capacity of the gel, a porous structure will arise by coalescing of the water-phase droplets into interconnected channels. The size of the pores depends on the polymerization conditions. On drying the porous sample, a transparent solid material is formed which takes on the original shape and structure on immersion in water.

After embedding in the living organism they do not show any irritating reactions as do other materials. Histological sections show successful encapsulation of the inserted object. Detailed biological results will be published in due course.

After more than four years of testing, these materials have now passed through the stage of application such as filling after enucleation of the eye. Promising results have also been obtained in experiments in other cases, for example, in manufacturing contact lenses, arteries, etc.

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Origin of Placental Trophoblast

In 1949 I published a hypothesis suggesting that the cyto- and syncytio-trophoblast were derived from the granulosa cells that surround the egg when it leaves the ovary; the argument, however, depended on indirect evidence, and although this indirect evidence has further accumulated it is not proposed to discuss it here[1].

Since then the work initiated by Barr and Bertram[2] on the nuclear sex chromatin has provided an opportunity to test the hypothesis. Obviously, if the trophoblast is fœtal its sex will always correspond with that of the fœtus; if it is maternal it should always be female. Workers have studied the normal placenta, chorionepithelioma and the hydatidiform mole. With respect to the normal placenta it must be recognized that my hypothesis refers only to the syncytium and the cytotrophoblast, and when this latter tissue disappears, only to the former. Other tissues in the placenta, such as those forming the vascular and supporting structures of the villus and cells derived from the outer covering of the blastocyst (chorionic epithelium), are undeniably of fœtal origin, and perhaps the last-named tissue also gives rise to cells that may equally in current usage be called cytotrophoblast. The only valid evidence, therefore, will result from sexing of the syncytium.

Park[3] worked on the embryos in the Carnegie Institution. His slides were old and not specially stained. He claimed that the trophoblast was always of the same sex as the fœtus; but when certain syncytial cells were female when they should have been male, he argued that these particular ones must have been derived from maternal stroma blending with developing trophoblast.

Klinger[4], Parmentier[5], Stevenson and McClarin[6], Bohle and Hienz[7] either did not examine the syncytium or found it unsuitable for study.

With respect to chorionepitheliomata Park[8], Oosterhuis and Levy[9] seem to have ignored the syncytium and the former suggests that trophoblast of chorionepitheliomata may not show any reliable sex difference.

Klinger and Ludwig[10] have recently described a more satisfactory method of staining the sex chromatin body and have applied it to the hydatidiform mole[11]. The results are interesting, for of nine moles examined all were female. The authors, in explanation, suggest (1) only female embryos develop moles; (2) there may have been a nuclear change, that is, the nuclei of the mole were polyploid, or (3) moles are derived from the maternal tissues. The first two explanations cannot be correct, for one of their moles developed with a male embryo, and Klinger and Schwarzacher[12] state that polyploid male nuclei (even $8n$) only occasionally show a sex chromatin mass and even so it is distinguishable from the average female diploid. The third explanation, and in my view the correct one, they do not elaborate.

In view of the results of experiments on homotransplantation it is strange that the syncytiotrophoblast is accepted so readily as of fœtal origin. Medawar[13] initiated the work showing that homotransplants do not take if the donor and recipient are not of identical genetic constitution, and the human mother and fœtus are generally not so identical. Tolerance can be produced in mice to homologous skin grafts only by manipulations in the recipient around the time of birth[14]. The human mother has not only more highly developed immunol-

ogical processes but also can in no sense be considered new-born, or a twin of the fœtus. Medawar suggests that the mammalian fœtus is usually protected from the consequences of homotransplantation as it has its strictly independent circulation. However, the human syncytiotrophoblast has a relationship with the maternal circulation that could not be closer. Algire *et al.*[15] can grow homografts indefinitely by inserting such into containers that prevent vascularization by the host and entry of cells. May the syncytium be Nature's analogue to Algire's millipore filter ?

It appears thus that the normal syncytium and the chorionepithelioma are difficult to sex but that the hydatidiform mole is female. It is considered that this supports my earlier suggestion that the syncytium is derived from granulosa cells, and its function is to protect the fœtus from the immunological processes of the mother.

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Influence of Filming and of Surface Texture on the Settlement of Marine Organisms

WHEN a well-cleaned surface is allowed to stand in sea water it becomes coated with a film in a few hours. The film increases in thickness for at least the first few days after it has formed. Several reports are to be found in the literature of a filmed surface being more favourable to settlement than a freshly cleaned one[1–4]. It has been widely assumed that this is true for all marine larvæ, since a permanent surface suitable for settlement is bound to collect a film in the sea. The extreme view has indeed been expressed that filming may be a necessary pre-requisite to the attachment of marine larvæ, although larvæ of some sedentary organisms have been observed to attach themselves to newly submerged surfaces before any visible film has formed[2].

A second assumption frequently made is that marine larvæ will attach themselves to a roughened surface more readily than to a smooth one.

Both these assumptions are being currently tested on a variety of invertebrate larvæ. Small rectangular pieces of chemically inert material are used, the upper surfaces of which are prepared in a series of grades of roughness from a shiny glossy finish to a coarsely ground surface. The surfaces are filmed for periods of 24 hr. or more and arranged in a factorial experiment around the periphery of a cylindrical dish containing larvæ, and slowly rotated in order to equalize such external influences as uneven illumination, to which the larvæ might be susceptible.

Table 1. NUMBER OF LARVÆ OF *Spirorbis borealis* AND OF *Bugula flabellata* SETTLING ON EQUAL AREAS OF PREPARED CHEMICALLY INERT SURFACES

Type of surface texture	*Spirorbis borealis*			*Bugula flabellata*		
	Filmed	Clean	Total	Filmed	Clean	Total
Glossy	826	23	849	175	967	1,142
Smooth but dull	273	239	512	93	1,327	1,420
Rough	1	0	1	88	960	1,048
Very rough	11	0	11	54	596	650
Totals	1,111	262	1,373	410	3,850	4,260

Our experiments, which will be fully reported elsewhere, have already shown that neither of the above generalizations is valid. The larvæ of many organisms settle preferentially on smooth rather than on rough surfaces, while others are indifferent to surface texture. Furthermore, although the majority of larvæ appear to choose a filmed surface, those of one species at least, *Bugula flabellata*, settle more readily on a clean unfilmed surface than on a filmed one. Moreover, we have verified that the film eschewed by *Bugula flabellata* was not a film of an accidentally unfavourable character, since it simultaneously attracted the larvæ of another animal, *Spirorbis borealis*, known for some time to prefer filmed surfaces[3]. The two kinds of larvæ in roughly similar numbers were placed together in a dish containing a series of surfaces, both filmed and unfilmed. As can be seen from the results in Table 1, larvæ of *Spirorbis* were attracted but those of *Bugula* were repelled by the film. Furthermore, *Spirorbis* larvæ clearly avoided the rougher surfaces, while *Bugula* larvæ were almost indifferent to surface texture.

It seems clear that a considerable diversity of behaviour towards films exists even in these simple ciliated larvæ; they seem able to respond both to the form of the surface and also to the presence or absence of a film overlying it. Further work may well show that many of them can distinguish qualitatively between films, as the larvæ of *Ophelia bicornis* appear to do[4]. No simple generalizations therefore seem applicable to the reactions of settling larvæ on filmed surfaces.

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Elminius modestus Darwin on the Isle of Cumbrae (Firth of Clyde)

THE distribution and rate of spread of the immigrant intertidal barnacle *Elminius modestus* Darwin in the British Isles and in the rest of north-west Europe have been summarized recently[1]. The most northerly published record is that of Connell[2], who found a single specimen of *Elminius* on Farland Point, Isle of Cumbrae, on March 7, 1955; the nearest published

records to this are those for Stranraer and Kirkcolm in Loch Ryan[1], about 55 miles to the south.

In view of the interest attached to the well-documented[1] rate of spread of this barnacle, search has been made at intervals since 1955 for further specimens on the Isle of Cumbrae; but none was seen until September 3, 1959, when two specimens were found on the exposed tip of Keppel Point, about 525 m. north-east of Connell's find. The two *Elminius*, which were left *in situ*, had settled about 3 m. apart, near mean tide-level, on rock among large *Balanus balanoides*; both specimens were small (3·5 and 3·6 mm. mean diameter, September 29) and presumably had settled during the summer of 1959.

Since it is believed that cross-fertilization is obligatory in *Elminius* and that in consequence individuals separated by a distance exceeding 5 cm. cannot breed[1], much larger settlements are required before the species can become fully established here.

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Mass Rearing of Barnacle Cyprids in the Laboratory

Interest in laboratory experiments on ships' fouling organisms is increasing, yet attempts to rear barnacle larvæ to cyprid stage have generally met with only limited success[1–6]. It therefore seems appropriate to place on record a reliable rearing method, which has been developed here to provide material in connexion with experiments on the use of ultrasonic antifouling equipment. The barnacle mostly used was *Elminius modestus*, as this important fouling organism produces nauplii all the year round. As a result of many series of experiments the following simple technique was evolved.

All sea water used is first pasteurized by heating to 60° C. and then cooled to room temperature. Masses of ripe embryos are dissected out of adult barnacles and placed in a dish of sea water on the side remote from the light. Stage I nauplii soon hatch from a proportion of the egg masses and swim rapidly to the lighted side of the dish, tending to leave behind Protozoa that may have been introduced with the egg masses. From the lighted side the nauplii are pipetted into 1 or 3 l. 'Pyrex' Berzelius beakers of sea water to give a larval density not exceeding 300 per l. *Skeletonema costatum* (Plymouth Collection No. 106), cultured in standard Erdschreiber medium[7], is used as food. Actively growing culture is added at a density of 200–500 cells per mm.[3] at the beginning of the experiment. The diatoms tend to settle to the bottom, but gentle stirring with a glass rod once a day is generally found to resuspend them sufficiently, particularly as the nauplii are able to feed on them while they are on the bottom. The vessels are covered and kept on a laboratory bench where they receive north daylight. No change of water or vessel is carried out and growth of the food plant usually makes good the quantity consumed, but excess food should always be present and more diatoms are added whenever necessary. Considerable fluctuations of temperature are tolerated. At a temperature of about 22° C. cyprids of *Elminius* appear after six days and have been obtained at all months of the year.

More than 90 per cent of the stage I nauplii eventually reach cyprid stage in such a culture and 80 per cent or more of these settle if provided with a suitable substratum. These figures contrast with the overall survival to the settled state of 16·3 and 12·7 per cent recently reported for individually reared larvæ of *Balanus eburneus* and *B. amphitrite* var. *denticulata*, respectively[4,5]. The main reasons for the failure of previous workers to obtain consistently high yields are probably contamination of cultures with other organisms, especially ciliates, overcrowding or stale or wrong food.

Although gentle bubbling of air from a glass jet has often been used to help keep the food suspended, careful experiments show that results with *Elminius* in bubbled vessels are no better than in stagnant cultures where satisfactory conditions have been established. There is thus no support for the idea recently put forward[8] that agitation of the water is necessary for ecdysis to be successful. Similarly, penicillin and streptomycin, which have been recommended in culturing oyster larvæ[9], do not produce significantly better results when added to healthy cultures, although they help in poor culture conditions where many larvæ have died.

Besides *Elminius* the cyprids of *Verruca stroemia*, *Chthamalus stellatus*, *Balanus amphitrite* var. *denticulata*, *B. perforatus*, *B. balanoides*, *Acasta spongites* and *Pyrgoma anglicum* have all been reared in large numbers. The larvæ of the two latter species are being described. Other plant cultures have been used as food with varying success and their relative values are being tested. Already it is clear that *Elminius* grows and survives better when fed on *Skeletonema* than on *Phaeodactylum*. Japanese workers have also found *Skeletonema* suitable as food for barnacle larvæ[10]. *Chthamalus stellatus*, however, though capable of being reared satisfactorily on the small flagellate, *Isochrysis galbana*, has not been reared successfully on *Skeletonema* and therefore seems to show a difference from Balanidae with which the fine setulation of the larval limbs in *Chthamalus* is probably correlated. The use of liver or egg extract as food[11] has not been tried, but would obviously need a procedure entailing frequent water changes to remove decomposing food.

I am grateful to the Marconi International Marine Communication Company, Limited, for financing this research, to Dr. Mary Parke of the Marine Laboratory, Plymouth, for supplying the plant cultures and to Prof. E. W. Knight-Jones for his advice and criticism.

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Terrestrial Ostracods of New Zealand

TERRESTRIAL ostracods have been known since 1953 from South Africa when Dr. R. F. Lawrence found specimens of *Mesocypris terrestris* Harding, in the humus of the Knysna forest[1]. Although there is no published record they have been found on several occasions in New Zealand, also in forest litter. Specimens from Leith Saddle, Dunedin, have recently been extracted alive by slowly drying out litter in a Berlese funnel, with a water-jacket maintained at a temperature of 40° C. and with the end leading into water, not alcohol. The temperature must be low to prevent the ostracods closing their valves and remaining immobile, and this is probably the reason why they have been found so infrequently. The animals are easily kept alive in a small dish lined with moist filter paper, and with a few fragments of humus to provide food and shelter.

The New Zealand specimens also appear to belong to the genus *Mesocypris* Daday, but differ in several respects from other described species. The valves of the shell are of equal size. Typical dimensions are: length, 0·56 mm., and height, 0·28 mm., the colouring being pale lemon unevenly distributed. Two specimens of the fifty which I have seen are considerably larger, being as much as 1·04 mm. long and 0·62 mm. high. The colour is also different, with patches of light brown. No males have yet been found. There is a marked dorso-ventral flattening, and the shell is moderately hairy. The appendages are broadly similar to those of *M. terrestris* with well-developed claws and a reduced number of setæ. A full description will be published later.

Locomotion is by means of the second antennæ and the third legs, both of which are armed with claws at the tips. An extra leg, used also for balance in steep places, is provided by the two halves of the furca, which act together. The animals are capable of moving up and down vertical slopes of humus, and one specimen was seen to climb several centimetres up the smooth sides of a glass dish. The first antennæ are used for sensory perception and balance, and with the setæ spread out explore objects ahead of the animal. They maintain a constant up and down motion, each alternating with the other. Sudden intense illumination causes the valves to close while bright light leads to rapid wandering in search of shelter. It is surprising to find apparently normal eyes, as Harding states that *M. terrestris*, like most litter-dwelling animals, appeared to be blind.

The Dunedin specimens were collected in ordinary forest litter from podocarp-broadleaf forest on Leith Saddle at an altitude of 1,200 ft. in places of no more than ordinary dampness. Experiments in the laboratory have shown that they are able to survive quite marked drying-out, although their activity decreases considerably and they tend to remain in holes in the humus. On the addition of more water they emerge and are capable of moving with great speed. On the other hand total immersion does not harm them and they will survive for at least a week. It is interesting that terrestrial Harpacticoid copepods were also found in the same habitat.

Other specimens known to me have been collected from Mount Arthur at 3,200 ft. (J. T. Salmon; 1946), Stewart Island (R. K. Dell and B. A. Holloway; 1955), Mount Egmont in sub-alpine forest (M. P. Büchler; 1954), at the foot of Mount Burnett, Nelson (R. Chapman; 1956), beech litter from Silverstream (J. D. Stout), Little Barrier Island 800 ft. (A. Chapman; 1959).

Further studies are in progress on the ecology and distribution of this New Zealand terrestrial ostracod.

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Ovaries of *Glossina morsitans*

CORRELATED with the viviparous type of reproduction found in the tsetse flies, the essential features of which were worked out as early as 1895 by Bruce[1], there is a great reduction in the number of ovarioles. Stuhlman[2] and Roubaud[3] studied the anatomy of the female reproductive organs and considered that each ovary consisted of a single ovariole, a view which has been perpetuated by every subsequent author. This communication, however, reports that in *Glossina morsitans* dissections have proved that each ovary contains, in fact, two ovarioles.

Dissections of teneral females were carried out in 0·9 per cent saline on a microscope slide. The ovarian sheaths were stripped off with a fine needle and the two ovarioles in each ovary released (Fig. 1, *a*, *b*). The slide was then drained of the excess saline and the ovaries mounted directly in a drop of polyvinyl-lactophenol containing a little lignin pink. This mountant produced a permanent preparation and avoided distortion of the ovarioles.

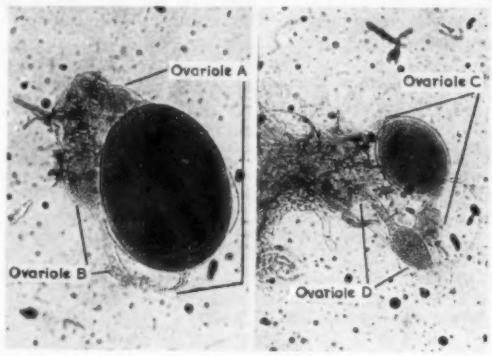

Fig. 1. (*a*) Right ovary of a 24-hr.-old *Glossina morsitans*, dissected to show the two ovarioles. (*b*) Left ovary, ditto

In a study of the ovulation cycle of female *G. palpalis*, Helen Mellanby[4] showed that the first egg is ovulated from the right ovary, and the subsequent eggs alternately from left and right ovaries throughout the reproductive life of the fly. The cycles in *G. morsitans* and *G. swynnertoni* have been shown to be the same[5]. In the light of the fact that there is a total of four ovarioles, however, the ovulation cycle in *Glossina* requires a more detailed study. Fig. 2 shows the four ovarioles completely removed from the ovaries of a 24-hr. old female *G. morsitans*. The most advanced egg follicle is in one (*A*) of the two ovarioles in the right ovary; the ovum and yolk occupy about the lower third of the follicle. The

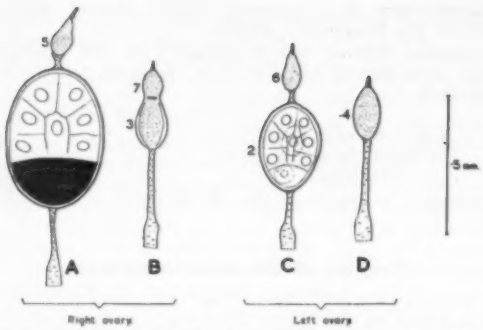

Fig. 2. The four ovarioles of a 24-hr.-old *G. morsitans*. Nos. 1–7 : follicles in the order of development

next most advanced follicle is in one of the ovarioles (*C*) in the left ovary. This follicle shows only the beginning of yolk deposition. The second ovariole in the right ovary (*B*) shows the early stages in the development of its first egg follicle and the second ovariole in the left ovary (*D*) shows only the germarium and no signs of follicle development. From this appearance of the ovarioles in the newly emerged female it would seem that the general outline of the ovulation cycle as described by Helen Mellanby is true but must now be modified. The first egg is probably ovulated from ovariole *A*, the second from *C* and the third and fourth from *B* and *D*, respectively. The fifth egg produced will develop in the second follicle in ovariole *A*.

Further studies of the ovulation cycle in *G. morsitans* are in progress and will include those with particular reference to the state of the follicular tubes after ovulation.

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VIROLOGY

Native Protein, an Immunizing Agent against Virus Infection

With several plant virus diseases, an infection, after becoming generalized, often results in apparent recovery of the plants, followed by an acquired immunity from reinfection[1]. The term, acquired immunity, has further been applied to the interference between virus strains in cross-protection tests.

The prevailing theory is that the virus particles are attached to 'acceptor points' in the plant cell[2]. Once these 'acceptors' are occupied no new virus particles of the same virus or related strains (requiring same 'acceptor') can multiply in the cell.

In this preliminary communication, a kind of immunization, apparently not due to antagonism of related strains, is reported.

Purified tobacco mosaic virus was prepared from tobacco plants, inoculated by a single lesion of common tobacco mosaic virus. The virus was purified by differential centrifugation[3]. This virus preparation, containing about 1 per cent tobacco mosaic virus, was degraded by Fraenkel-Conrat's method, using 67 per cent acetic acid[4]. Any undegraded virus was removed by ultra-centrifugation. The native protein obtained was tested on *Nicotiana glutinosa* L., and found to be free of virus particles.

In the first series of experiments half sides of detached leaves of *N. glutinosa* were inoculated with this native protein solution, freshly prepared, using 'Carborundum' as an abrasive. The other half of the leaf was similarly rubbed with water. The leaves were kept on moist filter paper, in Petri dishes. After varying intervals the whole leaves were rubbed with a diluted solution of tobacco mosaic virus; 'Carborundum' was again used. After 3–4 days lesions appeared and were counted separately for each half-side. Results, a mean of eight replicates, are summarized in Table 1.

Table 1. Effect of Native Protein on Tobacco Mosaic Virus Infection in Detached Leaves (Average of Eight Replicates)

No. of inoculations with native protein	Tobacco mosaic virus inoculation, days after last protein treatment	No. of lesions in protein treated/untreated half-leaves	Ratios of lesion numbers in protein treated/untreated half-leaves (average)
1	1 hr.	52 : 46	1·15
1	1 day	52 : 56	0·96
1	2 ,,	60 : 81	0·79*
1	4 ,,	36 : 59	0·61*
2 (second after 24 hr.)	2 ,,	69 : 120	0·59*
2 (second after 24 hr.)	4 ,,	16 : 57	0·36†
2 (second after 2 days)	2 ,,	19 : 47	0·39†
2 (second after 2 days)	4 ,,	31 : 56	0·57*

* Significant at 0·05 per cent. †, Significant at 0·01 per cent.

Due to the extent of variation in lesion numbers within the replicates, the ratio between the two leaf half-sides was found to be much more constant, and was therefore used for statistical analysis.

As shown, no effect was found if the leaves were inoculated with tobacco mosaic virus either immediately or 24 hr. after the virus protein had been applied. The effect of the protein is more pronounced from two doses than from one, and increased somewhat after 4 days as compared with after 2 days.

In a second series similar results were obtained. The number of lesions and the ratio between treated and untreated half-sides varied; in some cases a ratio of even 1 : 9 in the protected leaf half-side was obtained.

In further experiments undetached leaves of *N. glutinosa* plants were treated. Each formula was tested on two plants—eight half-sides altogether. Protein and tobacco mosaic virus inoculations were conducted as described above. Results, a mean of eight replicates, are summarized in Table 2.

It should be added that the absolute number of lesions, as well as the ratio values, should be regarded cautiously, as the susceptibility of leaves and plants may vary. Nevertheless the striking effect of the proteins is clearly evident.

These preliminary results suggest the formation of 'protective substances' within the plant. As no

Table 2. Effect of Native Protein on Tobacco Mosaic Virus Infection in Undetached Leaves (Average of Eight Replicates)

No. of inoculations with native protein	Tobacco mosaic inoculation days after last protein treatment	No. of lesions in protein treated/ untreated half-leaves	Ratios of lesion numbers in protein treated/untreated half-leaves (average)
1	1 hr.	38 : 37	1·12
1	1 day	46 : 39	1·20
1	2 days	30 : 44	0·69*
2 (second after 24 hr.)	2 ,,	39 : 49	0·64*
2 (second after 2 days)	4 ,,	38 : 76	0·56†

* Significant at 0·05 per cent. † Significant at 0·01 per cent.

result was obtained when the protein inoculations were done immediately before tobacco mosaic virus infection, direct inhibiting effect does not seem to be the cause. A direct inhibiting effect of native protein, if mixed with tobacco mosaic virus before inoculation, is known to occur, but only in concentrated doses[5]. With dilute solutions as used in our experiments no inhibition could be observed[6].

The increase in the depressing effect observed after two protein applications, and after 4 days, as compared to 1 and 2 days, seems to suggest an analogy with antibody formation in animals.

Attachment of the protein to 'acceptor points' appears unlikely, as the protein itself does not reproduce. After infection the virus multiplies and establishes itself thoroughly before cross-protection is effective[7]. The initial amount of protein applied would, therefore, not be sufficient to produce this effect.

A full report will be published elsewhere.

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Multiplication of Vaccinia Virus in Serum-free and Serum-containing Cell Cultures

The use of tissue culture cells for the production of virus preparations and vaccines is increasing and will gain prominence in the future. A report has recently appeared concerning the possible use of cell cultures for the standardization of smallpox vaccines[1]. At present the cell lines used for the production of virus vaccines are grown in media which contain of necessity serum of one species or another[2]. Several cell lines which grow in media free of serum have been reported[3], and the advantages that these cells might have for vaccine production are obvious. Their use, however, could well be limited by their ability to support virus growth to a high titre. This report describes a comparison of vaccinia growth in serum-free and serum-containing mouse lung cells.

The cells used for growth studies were isolated from (new-born) white Swiss mice by the method of Younger[4]. The serum-containing line proliferated rapidly on a growth medium consisting of 10 per cent horse serum and 90 per cent medium 199[5]. The serum-free daughter culture was adapted to rapid growth on a medium in which the serum was replaced by 1 per cent Difco 'Bactopeptone' and 50–100 mgm. per cent dextrose[3]. The serum-containing line has been designated *MSI*, and the peptone line *MPDI*. At the time of use the *MSI* line was in its one hundred and twelfth passage ($2\frac{1}{2}$ yr.) and the *MPDI* line in its sixty-fifth passage in peptone ($1\frac{3}{4}$ yr.).

The vaccinia virus stock preparation strain *IDH–E*[6], which was normally maintained in chick embryos, was passed once in *MSI* or *MPDI* cells prior to experimental use. This was done to circumvent the possibility that any of the chick embryo protein might be carried along as a stimulatory factor when the virus was added to the cell cultures.

The *MSI* or *MPDI* cells were inoculated into a volume of 15 ml. of medium in 'Pyrex' milk dilution bottles, the total number of cells being approximately 3×10^7 (2×10^5 cells per ml.). After 48 hr. of incubation at 37° C. the cells were inoculated with 0·1 millilitre of a $1{\cdot}0 \times 10^{-3}$ dilution of the stock virus preparation. Immediately after inoculation, and every 24 hr. for a total of 8 days, 0·4 ml. of the supernatant fluid was harvested and stored frozen. Following the final harvest the virus was titrated by inoculating 0·1 ml. of a ten-fold dilution into each of 4, 16 mm. × 150 mm. test-tubes containing $2{\cdot}5 \times 10^5$ cells and 1 ml. of medium. The cell types used in this titration were, *MPDI*, *MSI* or N.C.T.C. clone 929 (strain *L*)[7]. The tubes were incubated at 37° C. at an angle of 5° and examined daily by use of a microscope. The titration end-point was considered as the final tube showing at least 20 per cent cell destruction within 10 days. The 50 per cent tissue culture infective dose ($TCID_{50}$) was then calculated by the method of Reed and Muench[8]. Plaques regularly appeared in 3–6 days in tubes which eventually showed the necessary cell destruction.

As an alternative method of titration, rabbits were inoculated intradermally with 0·1 ml. of the same ten-fold dilutions of virus. The end-point in this titration was taken as the highest dilution of virus eliciting an inflammatory lesion which progressed through pustulation and scar formation.

The results of a typical growth study are shown in Table 1. Only the titre of the 5-day harvest is shown, since the rate of growth was approximately the same in both cell lines. The maximum titre was usually reached on the fifth or sixth day, and the peak titre was maintained in both cell lines for at least 24 hr. The greatest amount of virus present in the peak titres in both cell lines averaged about 10 times higher than that previously shown with the Levaditi strain of vaccinia in serum-containing mouse lung cells[9].

It is apparent that cells grown in the absence of serum or its derivatives are able to support vaccinia multiplication to about the same degree as serum-containing lines. It also appears that serum-free cells are not as sensitive for the determination of

Table 1. Multiplication of Vaccinia in Serum-free and Serum-containing Cell Lines. Maximum Titres

Virus grown in :	Negative log$_e$ $TCID_{50}$ titre of virus titrated in : *MSI*	*MPDI*	*L*	Rabbits
MSI (serum)	6·3	6·0	6·5	6·3
MPDI (serum-free)	6·0	5·5	6·0	6·2

vaccinia infective titres as are serum-containing lines. This, however, may be related to the decreased stability of small numbers of virus particles in a non-serum-containing medium. The important point in this study is that the final titre of vaccinia is similar in both cell lines. After four passages in serum-free cells the virus has not yet shown a greatly diminished infectivity for rabbits as has been reported in other serum-containing lines[1].

Experiments are planned using other viruses in this same cell system. We are indebted to Dr. Wm. Cassel for provision of the vaccinia virus used in these studies.

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SCIENTIFIC METHOD

A Proposal to replace Belief by Method in the Pre-mensural Sciences

Not only in his posthumous work, "Fundamental Theory"[1], but also in many of his earlier writings, Eddington was concerned to define the mathematical structure of what he called a 'measurable', and to distinguish a measurable from an observable, and an observable from the most primitive entity, which he regarded as resembling a geometrical point except that, instead of having position only (a far from primitive attribute), it has existence (or non-existence) only.

Eddington's proposed algebraic representations of these three concepts can be translated into the language of communication theory as follows: an entity has two characteristics, existence and non-existence, and so can be represented by one binomial unit of information (bit). An observable is represented by two entities and has four characteristics, while a measurable is represented by two observables (or four entities) and has sixteen characteristics. This means that an entity has one way of not existing out of two possibilities, an observable three out of four, and a measurable fifteen out of sixteen.

We have devised[2] an information model which represents the concept of a 'comparable', intermediate between an observable and a measurable. A comparable is represented by three bits of information, has eight characteristics, and accordingly it has seven ways of not existing within this universe of discourse.

The information structure (called the O-structure, because it incorporates what is necessary for measuring or 'observing'—Eddington's Z) is a hierarchical structure. If the whole structure consists of n bits there will be $\frac{1}{2}n$ in the ground-level, $\frac{1}{4}n$ in the next, and so on for the L levels, where $L = \log_2 n$, the number of bits in any level, l, being $n/2^l$.

So long as a science is based upon measurables, its primitives must be measurable things, that is, atoms, but if a science is based upon comparables, this allows comparisons to be made between an observable entity, such as a semantic relationship with an observable, such as a physiological response. Luria's work[3] along these lines makes meaning observable, but not measurable in the physical sense, and so, in principle, separates meaning from knowledge in just the same way as the cybernetic engineer separates information from meaning.

It is suggested that these separations now make it possible to formulate a scientific methodology of pre-mensural science, based upon similarity and comparable with the formal methodologies of dynamics and wave mechanics in the mensural sciences.

We represent the primitive entity, first by the binomial unit of information for ideal representation, and secondly as a two-way switch, in order that this ideal representation can be exemplified, at least in principle, by a cybernetic artefact. This double representation has been found capable of representing some characteristics of neural events, but owing to the great complexity both of these events and of experienced events (memories) it is not to be expected that detailed isomorphism can be established. However, it is easy to show how a connected system of switches can represent what is required, at least in a general way. If we start by assuming that all the connected switches have an equal probability of being open or closed, that is, a half-probability of existence, this will represent a system in which information or probability flow is everywhere maximal and uniform. If we now consider that some instability creates a linked succession of switches in the closed position, this will represent maximal existence and zero information. Such a linking inevitably produces a number of informationally isolated systems of zero existence and zero information; such systems represent events that are not being experienced. If we adopt Eddington's proposal that numbers between 0 and 1 represent degrees of statistical existence (phase) this allows us to suppose slight imperfections in our two types of system. The linking of chains of switches in the state of maximal existence may be imperfect (synaptic gaps), and as regards the isolated events that provide the possibility of recognition, these may be supposed to accumulate sufficient information by similarity to reach what might be called a 'semantic threshold' as a unit entity. It is such a unit entity that we envisage as the third element of a comparable, the other two being provided, in the case of perception, by two micro-neural events or quanta that together constitute an observable.

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FORTHCOMING EVENTS

Monday, January 11

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. G. G. Gouriet and Mr. G. F. Newell: "A Quadrature Network for Generating Vestigial-Sideband Signals"; Prof. D. G. Tucker: "The Input Impedance of Rectifier Modulators"; Mr. D. P. Howson and Prof. D. G. Tucker: "Rectifier Modulators with Frequency-Selective Terminations, with particular reference to the Effect of Even-Order Modulation Products".

SOCIETY OF CHEMICAL INDUSTRY, SURFACE ACTIVITY GROUP (joint meeting with the OILS AND FATS GROUP, at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. C. G. Sumner: "Emulsions in Theory and Practice".

MANCHESTER LITERARY AND PHILOSOPHICAL SOCIETY (in the Reynolds Hall, Manchester College of Science and Technology, Sackville Street, Manchester), at 5.45 p.m.—Dr. G. E. R. Deacon, F.R.S.: "Scientific Exploration of the Ocean" (Clayton Memorial Lecture).

INSTITUTE OF PHYSICS (at 47 Belgrave Square, London, S.W.1), at 6 p.m.—Prof. J. G. Wilson: "The Problem of Primary Cosmic Radiation".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. W. H. Melbourne and Mr. P. Garrard: "Explorations on the Bolivian–Peruvian Border".

Tuesday, January 12

INSTITUTION OF THE RUBBER INDUSTRY (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 5.30 p.m.—Mr. J. Westhead: "Chemical Plant Lining". 7 p.m.—Dr. J. N. Milne: "Hypalon Coatings".

Wednesday, January 13

SOCIETY FOR APPLIED BACTERIOLOGY (in the Barnes Lecture Theatre, Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 9.45 a.m.—Winter Meeting. At 2.15 p.m. (joint meeting with the MICROBIOLOGY GROUP of the SOCIETY OF CHEMICAL INDUSTRY)—Symposium on "Methods for the Evaluation of the Antibacterial Activity of Surface Active Compounds".

BONE AND TOOTH SOCIETY (in the Cuthbert Wallace Room, Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2), at 11 a.m.—Meeting on "Radioactive Materials and the Skeleton".

PHYSICAL SOCIETY, LOW TEMPERATURE GROUP (in the Lecture Theatre of the Royal School of Mines, Prince Consort Road, London, S.W.7), at 4.30 p.m.—Dr. Audrey U. Smith: "Effects of Sub-Zero Temperatures on Living Cells, Tissues and Animals".

INSTITUTION OF ELECTRICAL ENGINEERS, SUPPLY SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. E. C. Rogers and Mr. D. J. Skipper: "Gaseous Discharge Phenomena in High-Voltage D.C. Cable Dielectrics".

SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. J. N. Wanklyn: "Corrosion Problems of High Temperature Pressurized Water Reactors".

BRITISH INSTITUTION OF RADIO ENGINEERS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Dr. G. T. Wright: "A Proposed Space-charge-limited Dielectric Triode".

WOMEN'S ENGINEERING SOCIETY (at "Hope House", 45 Great Peter Street, London, S.W.1), at 7 p.m.—Mr. B. C. Godbold: "Health Physics and Nuclear Power".

Wednesday, January 13—Thursday, January 14

BRITISH COAL UTILIZATION RESEARCH ASSOCIATION (at the Research Station, Randalls Road, Leatherhead, Surrey)—Symposium on "Aerodynamic Capture of Particles".

Thursday, January 14

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Mr. R. L. E. Dreschfield: "The Preservation of Game in East Africa".

INSTITUTION OF ELECTRICAL ENGINEERS, UTILIZATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. I. F. Davies and Mr. D. Dunthorne: "The Application of Power Transistors to the Operation of Gas Discharge Lamps from Direct Current Supplies".

CHEMICAL SOCIETY (in the Lecture Theatre, The Royal Institution, Albemarle Street, London, W.1), at 7.30 p.m.—Prof. C. Kemball: "Progress in the Study of Heterogeneous Catalysis" (Tilden Lecture).

Friday, January 15

INSTITUTE OF NAVIGATION (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 5.15 p.m.—Mr. J. F. Green and Mr. A. P. Glenny: "Heading Definition in Commercial Aircraft".

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. A. H. Beckett: "Recent Developments in Analgesics".

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. C. V. Dadd: "Biological Factors in Agricultural Aviation".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT BIOCHEMIST, Basic Grade (with a science degree, preferably with honours in biochemistry or chemistry, or the A.R.I.C.)—The House Governor, The General Hospital, Birmingham 4 (January 16).

PHYSICIST (Basic Grade), for duties in connexion with radiotherapy and isotopes—The Personnel Officer, St. Thomas's Hospital, London, S.E.1 (January 16).

VICE-PRINCIPAL (with high academic and/or professional qualifications with substantial administrative experience of a relevant kind, for example, in education, industrial research or other professional work)—The Registrar, College of Advanced Technology, Gosta Green, Birmingham 4 (January 16).

BIOLOGIST, Assistant Experimental Officer grade (with a pass degree in a biological subject or equivalent), for work on plant virus diseases—The Secretary, Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, Sussex (January 20).

LECTURER IN EDUCATION—The Principal, Borough Road College, Isleworth, Middlesex (January 23).

LECTURER (Grade II) IN PURE MATHEMATICS—The Registrar, The University, Bristol 8 (January 25).

TECHNICAL EDITOR (Experimental Officer, preferably under 31, with at least H.N.C. or Inter-B.Sc. or equivalent, some knowledge of terms used in the fields of fuel and power, the ability to translate French and German, and preferably a knowledge of Russian) at the Ministry of Power, H.Q., London, for abstracting scientific articles; preparations of surveys of current literature; briefs and memoranda on fuel and power subjects—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting A.572/9A (January 29).

LECTURER IN MATHEMATICS at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand, January 31).

LECTURER (with some industrial or research experience, and preferably some teaching experience) IN PHYSICS—The Registrar, Royal Technical College, Salford 5, Lancs (January 31).

SENIOR LECTURER or LECTURER (preferably with a medical degree) IN THE DEPARTMENT OF PATHOLOGY, University College of the West Indies—The Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W.C.1 (February 8).

LECTURER (with a special interest in plant physiology) IN BOTANY at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 10).

RESEARCH FELLOW (with M.Sc. or equivalent, with a special knowledge and/or experience of agronomy of tropical crops) IN TROPICAL CROP PRODUCTION at the Agricultural Research Station, University College of Ghana, Kade, in the forest region of Ghana—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 10).

RESEARCH FELLOWS (2) (with research experience in animal virology or in biochemistry) IN THE DEPARTMENT OF MICROBIOLOGY, John Curtin School of Medical Research, Australian National University, to work on either biological or biochemical aspects of animal virology—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (February 12).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN PHYSICS, CHEMISTRY, BIOCHEMISTRY, ENGINEERING, METALLURGY and PHARMACOLOGY or any related subjects—The Registrar, The University, Liverpool (February 13).

UNIVERSITY LIBRARIAN—The Registrar (Room 22, O.R.B.), The University, Reading (February 13).

PROFESSOR AND CHAIRMAN (with extensive experience in both teaching and research, preferably in the large animal field) OF THE DEPARTMENT OF ANIMAL SCIENCE—The Dean, Faculty of Agriculture, McGill University, Macdonald College, P.Q., Canada (February 15).

SENIOR LECTURER IN GEOGRAPHY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (February 15).

SENIOR LECTURER IN ARCHÆOLOGY AND ETHNOLOGY at the University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa, February 15).

UNIVERSITY TUTOR or ASSISTANT LECTURER (medical and/or science graduate) IN MICROBIOLOGY—The Secretary, The Queen's University, Belfast (February 15).

PROFESSOR OF INORGANIC CHEMISTRY—The Registrar, University Registry, Oxford (February 18).

LECTURER/SENIOR LECTURER (with good academic qualifications in electrical engineering, experience in the power field, and a working knowledge of electronic techniques) IN ELECTRICAL ENGINEERING at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, February 19).

PASTURE UTILIZATION OFFICER (honours graduate in science or agricultural science (or equivalent qualifications), with some years post-graduate research experience and training in animal physiology and nutrition) IN THE DIVISION OF PLANT INDUSTRY, Commonwealth Scientific and Industrial Research Organization, Canberra, A.C.T., Australia, to participate in the current programme of investigation which seeks to understand the plant, animal and plant–animal factors in normal grazing systems, by studying the animal factors affecting the nutrition demands of the ewe (and lamb) at pasture, of the ability, or otherwise, of the pasture to satisfy these demands—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 130/391 (February 26).

PLANT BREEDER (honours graduate in science or agricultural science (or equivalent qualifications) with two or more years relevant post-graduate research experience) IN THE DIVISION OF PLANT INDUSTRY,

vaccinia infective titres as are serum-containing lines. This, however, may be related to the decreased stability of small numbers of virus particles in a non-serum-containing medium. The important point in this study is that the final titre of vaccinia is similar in both cell lines. After four passages in serum-free cells the virus has not yet shown a greatly diminished infectivity for rabbits as has been reported in other serum-containing lines[1].

Experiments are planned using other viruses in this same cell system. We are indebted to Dr. Wm. Cassel for provision of the vaccinia virus used in these studies.

Robert W. Pumper
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[1] Cutchins, E., and Warren, J., *Proc. Soc. Exp. Biol. Med.*, **97**, 456 (1958).
[2] Hilleman, M. R., *et al.*, *J. Amer. Med. Assoc.*, **163**, 4 (1957). Salk, J. E., *ibid.*, **158**, 1239 (1955).
[3] McQuilkin, W. T., Evans, V. J., and Earle, W. R., *J. Nat. Cancer Inst.*, **19**, 885 (1957). Waymouth, C., *ibid.*, **22**, 1003 (1959). Pumper, R. W., *Science*, **128**, 363 (1958).
[4] Younger, J. S., *Proc. Soc. Exp. Biol. Med.*, **85**, 202 (1954).
[5] Morgan, J. F., Morton, H. J., and Parker, R. C., *Proc. Soc. Exp. Biol. Med.*, **73**, 1 (1950).
[6] Cassel, W. A., *Virol.*, **3**, 514 (1957).
[7] Sanford, K. K., Earle, W. R., and Likely, G. D., *J. Nat. Cancer Inst.*, **9**, 229 (1948).
[8] Reed, L. J., and Muench, H., *Amer. J. Hyg.*, **27**, 493 (1938).
[9] Pumper, R. W., *Amer. J. Hyg.*, **69**, 79 (1959).

SCIENTIFIC METHOD

A Proposal to replace Belief by Method in the Pre-mensural Sciences

Not only in his posthumous work, "Fundamental Theory"[1], but also in many of his earlier writings, Eddington was concerned to define the mathematical structure of what he called a 'measurable', and to distinguish a measurable from an observable, and an observable from the most primitive entity, which he regarded as resembling a geometrical point except that, instead of having position only (a far from primitive attribute), it has existence (or non-existence) only.

Eddington's proposed algebraic representations of these three concepts can be translated into the language of communication theory as follows: an entity has two characteristics, existence and non-existence, and so can be represented by one binomial unit of information (bit). An observable is represented by two entities and has four characteristics, while a measurable is represented by two observables (or four entities) and has sixteen characteristics. This means that an entity has one way of not existing out of two possibilities, an observable three out of four, and a measurable fifteen out of sixteen.

We have devised[2] an information model which represents the concept of a 'comparable', intermediate between an observable and a measurable. A comparable is represented by three bits of information, has eight characteristics, and accordingly it has seven ways of not existing within this universe of discourse.

The information structure (called the O-structure, because it incorporates what is necessary for measuring or 'observing'—Eddington's Z) is a hierarchical structure. If the whole structure consists of n bits there will be $\frac{1}{2}n$ in the ground-level, $\frac{1}{4}n$ in the next, and so on for the L levels, where $L = \log_2 n$, the number of bits in any level, l, being $n/2^l$.

So long as a science is based upon measurables, its primitives must be measurable things, that is, atoms, but if a science is based upon comparables, this allows comparisons to be made between an observable entity, such as a semantic relationship with an observable, such as a physiological response. Luria's work[3] along these lines makes meaning observable, but not measurable in the physical sense, and so, in principle, separates meaning from knowledge in just the same way as the cybernetic engineer separates information from meaning.

It is suggested that these separations now make it possible to formulate a scientific methodology of pre-mensural science, based upon similarity and comparable with the formal methodologies of dynamics and wave mechanics in the mensural sciences.

We represent the primitive entity, first by the binomial unit of information for ideal representation, and secondly as a two-way switch, in order that this ideal representation can be exemplified, at least in principle, by a cybernetic artefact. This double representation has been found capable of representing some characteristics of neural events, but owing to the great complexity both of these events and of experienced events (memories) it is not to be expected that detailed isomorphism can be established. However, it is easy to show how a connected system of switches can represent what is required, at least in a general way. If we start by assuming that all the connected switches have an equal probability of being open or closed, that is, a half-probability of existence, this will represent a system in which information or probability flow is everywhere maximal and uniform. If we now consider that some instability creates a linked succession of switches in the closed position, this will represent maximal existence and zero information. Such a linking inevitably produces a number of informationally isolated systems of zero existence and zero information; such systems represent events that are not being experienced. If we adopt Eddington's proposal that numbers between 0 and 1 represent degrees of statistical existence (phase) this allows us to suppose slight imperfections in our two types of system. The linking of chains of switches in the state of maximal existence may be imperfect (synaptic gaps), and as regards the isolated events that provide the possibility of recognition, these may be supposed to accumulate sufficient information by similarity to reach what might be called a 'semantic threshold' as a unit entity. It is such a unit entity that we envisage as the third element of a comparable, the other two being provided, in the case of perception, by two micro-neural events or quanta that together constitute an observable.

C. C. L. Gregory

Institute for the Study of Mental Images,
Gally Hill,
Church Crookham,
Hants.

[1] Eddington, A. S., "Fundamental Theory" (Camb. Univ. Press, 1946).
[2] *Com. Inst. Study Mental Images*. **1**, Part 1, 1 (1957). Gregory, C. C. L., and Kohsen, A., "The O-Structure: an Introduction to Psychophysical Cosmology" (I.S.M.I., 1959).
[3] Luria, A. R., and Vinogradova, O. S., *Brit. J. Psychol.*, **50**, 2, 89 (1959).

FORTHCOMING EVENTS

Monday, January 11

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. G. G. Gouriet and Mr. G. F. Newell: "A Quadrature Network for Generating Vestigial-Sideband Signals"; Prof. D. G. Tucker: "The Input Impedance of Rectifier Modulators"; Mr. D. P. Howson and Prof. D. G. Tucker: "Rectifier Modulators with Frequency-Selective Terminations, with particular reference to the Effect of Even-Order Modulation Products".

SOCIETY OF CHEMICAL INDUSTRY, SURFACE ACTIVITY GROUP (joint meeting with the OILS AND FATS GROUP, at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. C. G. Sumner: "Emulsions in Theory and Practice".

MANCHESTER LITERARY AND PHILOSOPHICAL SOCIETY (in the Reynolds Hall, Manchester College of Science and Technology, Sackville Street, Manchester), at 5.45 p.m.—Dr. G. E. R. Deacon, F.R.S.: "Scientific Exploration of the Ocean" (Clayton Memorial Lecture).

INSTITUTE OF PHYSICS (at 47 Belgrave Square, London, S.W.1), at 6 p.m.—Prof. J. G. Wilson: "The Problem of Primary Cosmic Radiation".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. W. H. Melbourne and Mr. P. Garrard: "Explorations on the Bolivian–Peruvian Border".

Tuesday, January 12

INSTITUTION OF THE RUBBER INDUSTRY (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 5.30 p.m.—Mr. J. Westhead: "Chemical Plant Lining". 7 p.m.—Dr. J. N. Milne: "Hypalon Coatings".

Wednesday, January 13

SOCIETY FOR APPLIED BACTERIOLOGY (in the Barnes Lecture Theatre, Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 9.45 a.m.—Winter Meeting. At 2.15 p.m. (joint meeting with the MICROBIOLOGY GROUP of the SOCIETY OF CHEMICAL INDUSTRY)—Symposium on "Methods for the Evaluation of the Antibacterial Activity of Surface Active Compounds".

BONE AND TOOTH SOCIETY (in the Cuthbert Wallace Room, Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2), at 11 a.m.—Meeting on "Radioactive Materials and the Skeleton".

PHYSICAL SOCIETY, LOW TEMPERATURE GROUP (in the Lecture Theatre of the Royal School of Mines, Prince Consort Road, London, S.W.7), at 4.30 p.m.—Dr. Audrey U. Smith: "Effects of Sub-Zero Temperatures on Living Cells, Tissues and Animals".

INSTITUTION OF ELECTRICAL ENGINEERS, SUPPLY SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. E. C. Rogers and Mr. D. J. Skipper: "Gaseous Discharge Phenomena in High-Voltage D.C. Cable Dielectrics".

SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. J. N. Wanklyn: "Corrosion Problems of High Temperature Pressurized Water Reactors".

BRITISH INSTITUTION OF RADIO ENGINEERS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Dr. G. T. Wright: "A Proposed Space-charge-limited Dielectric Triode".

WOMEN'S ENGINEERING SOCIETY (at "Hope House", 45 Great Peter Street, London, S.W.1), at 7 p.m.—Mr. B. C. Godbold: "Health Physics and Nuclear Power".

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BRITISH COAL UTILIZATION RESEARCH ASSOCIATION (at the Research Station, Randalls Road, Leatherhead, Surrey)—Symposium on "Aerodynamic Capture of Particles".

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ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Mr. R. L. E. Dreschfield: "The Preservation of Game in East Africa".

INSTITUTION OF ELECTRICAL ENGINEERS, UTILIZATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. I. F. Davies and Mr. D. Dunthorne: "The Application of Power Transistors to the Operation of Gas Discharge Lamps from Direct Current Supplies".

CHEMICAL SOCIETY (in the Lecture Theatre, The Royal Institution, Albemarle Street, London, W.1), at 7.30 p.m.—Prof. C. Kemball: "Progress in the Study of Heterogeneous Catalysis" (Tilden Lecture).

Friday, January 15

INSTITUTE OF NAVIGATION (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 5.15 p.m.—Mr. J. F. Green and Mr. A. P. Glenny: "Heading Definition in Commercial Aircraft".

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. A. H. Beckett: "Recent Developments in Analgesics".

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. C. V. Dadd: "Biological Factors in Agricultural Aviation".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT BIOCHEMIST, Basic Grade (with a science degree, preferably with honours in biochemistry or chemistry, or the A.R.I.C.)—The House Governor, The General Hospital, Birmingham 4 (January 16).

PHYSICIST (Basic Grade), for duties in connexion with radiotherapy and isotopes—The Personnel Officer, St. Thomas's Hospital, London, S.E.1 (January 16).

VICE-PRINCIPAL (with high academic and/or professional qualifications with substantial administrative experience of a relevant kind, for example, in education, industrial research or other professional work)—The Registrar, College of Advanced Technology, Gosta Green, Birmingham 4 (January 16).

BIOLOGIST, Assistant Experimental Officer grade (with a pass degree in a biological subject or equivalent), for work on plant virus diseases—The Secretary, Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, Sussex (January 20).

LECTURER IN EDUCATION—The Principal, Borough Road College, Isleworth, Middlesex (January 23).

LECTURER (Grade II) IN PURE MATHEMATICS—The Registrar, The University, Bristol 8 (January 25).

TECHNICAL EDITOR (Experimental Officer, preferably under 31, with at least H.N.C. or Inter-B.Sc. or equivalent, some knowledge of terms used in the fields of fuel and power, the ability to translate French and German, and preferably a knowledge of Russian) at the Ministry of Power, H.Q., London, for abstracting scientific articles; preparations of surveys of current literature; briefs and memoranda on fuel and power subjects—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting A.572/9A (January 29).

LECTURER IN MATHEMATICS at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand, January 31).

LECTURER (with some industrial or research experience, and preferably some teaching experience) IN PHYSICS—The Registrar, Royal Technical College, Salford 5, Lancs (January 31).

SENIOR LECTURER or LECTURER (preferably with a medical degree) IN THE DEPARTMENT OF PATHOLOGY, University College of the West Indies—The Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W.C.1 (February 8).

LECTURER (with a special interest in plant physiology) IN BOTANY at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 10).

RESEARCH FELLOW (with M.Sc. or equivalent, with a special knowledge and/or experience of agronomy of tropical crops) IN TROPICAL CROP PRODUCTION at the Agricultural Research Station, University College of Ghana, Kade, in the forest region of Ghana—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 10).

RESEARCH FELLOWS (2) (with research experience in animal virology or in biochemistry) IN THE DEPARTMENT OF MICROBIOLOGY, John Curtin School of Medical Research, Australian National University, to work on either biological or biochemical aspects of animal virology—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (February 12).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN PHYSICS, CHEMISTRY, BIOCHEMISTRY, ENGINEERING, METALLURGY and PHARMACOLOGY or any related subjects—The Registrar, The University, Liverpool (February 13).

UNIVERSITY LIBRARIAN—The Registrar (Room 22, O.R.B.), The University, Reading (February 13).

PROFESSOR AND CHAIRMAN (with extensive experience in both teaching and research, preferably in the large animal field) OF THE DEPARTMENT OF ANIMAL SCIENCE—The Dean, Faculty of Agriculture, McGill University, Macdonald College, P.Q., Canada (February 15).

SENIOR LECTURER IN GEOGRAPHY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (February 15).

SENIOR LECTURER IN ARCHÆOLOGY AND ETHNOLOGY at the University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa, February 15).

UNIVERSITY TUTOR or ASSISTANT LECTURER (medical and/or science graduate) IN MICROBIOLOGY—The Secretary, The Queen's University, Belfast (February 15).

PROFESSOR OF INORGANIC CHEMISTRY—The Registrar, University Registry, Oxford (February 18).

LECTURER/SENIOR LECTURER (with good academic qualifications in electrical engineering, experience in the power field, and a working knowledge of electronic techniques) IN ELECTRICAL ENGINEERING at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, February 19).

PASTURE UTILIZATION OFFICER (honours graduate in science or agricultural science (or equivalent qualifications), with some years post-graduate research experience and training in animal physiology and nutrition) IN THE DIVISION OF PLANT INDUSTRY, Commonwealth Scientific and Industrial Research Organization, Canberra, A.C.T., Australia, to participate in the current programme of investigation which seeks to understand the plant, animal and plant–animal factors in normal grazing systems, by studying the animal factors affecting the nutrition demands of the ewe (and lamb) at pasture, of the ability, or otherwise, of the pasture to satisfy these demands—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 130/391 (February 26).

PLANT BREEDER (honours graduate in science or agricultural science (or equivalent qualifications) with two or more years relevant post-graduate research experience) IN THE DIVISION OF PLANT INDUSTRY,

Commonwealth Scientific and Industrial Research Organization, Canberra, A.C.T., Australia, to collaborate with the established team of geneticists and plant breeders, and to assist in the extension of the current programme of studies on pasture plants by initiating independent studies on the breeding of pasture plants—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 130/403 (February 26).

Chair of Botany, tenable in the Newcastle Division of the University of Durham—The Registrar, University Office, 46 North Bailey, Durham (February 29).

Chair of Geography in the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand, February 29).

Chemist (with a good honours degree, experience in crop and soil analysis, and an interest in field experimentation), to investigate anion–cation relationships in soils and crop nutrition—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (February 29).

Imperial Chemical Industries Research Fellows in Chemistry, Biological Chemistry, Engineering, Pharmacology, Physics, or related subjects—The Registrar, The University, Bristol (March 1).

Imperial Chemical Industries Research Fellow in Chemistry or Physics—The Secretary, The University, Exeter (March 7).

Imperial Chemical Industries Research Fellows in Chemistry, Physiological Chemistry, Agricultural Chemistry, Agricultural Botany, Horticulture, Microbiology, Geology, Physics, or related subjects—The Registrar (Room 22, O.R.B.), The University, Reading (March 7).

Assistant, Grade B (with a good degree in chemistry and preferably some teaching experience) to teach Chemistry to G.C.E. Advanced and Part 1 B.Sc. level—The Clerk to the Governors, South-West Essex Technical College, Forest Road, Walthamstow, London, E.17.

Assistant Master (bachelor) to teach Mathematics—The Headmaster, Wrekin College, Wellington, Salop.

Assistant or Associate Professor (Ph.D. or equivalent) in the Soil Science Department, for duties which will include research, teaching and extension in the field of soil chemistry and soil fertility—Head of the Soil Science Department, University of Alberta, Edmonton, Alberta, Canada.

Biochemist (with a first- or second-class honours degree) in the Biochemistry Department, for duties mainly concerned with radioactive isotopes, including thyroid hormone synthesis and utilization—The Secretary, Glasgow Royal Infirmary, 54 Castle Street, Glasgow, C.4.

Chief Technician in the Department of Chemistry, for duties which will include the supervision of laboratories and technical staff, as well as responsibilities for furnishing and equipping new laboratories planned for completion in 1962—The Registrar, The University, Liverpool 3, quoting Ref. No. 240N.

Editorial Assistant (science graduate, preferably in biochemistry or chemistry, and preferably with some scientific editorial experience) at the Editorial Office of the *Biochemical Journal*—The Secretary to the Editorial Board, *Biochemical Journal*, The Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.1.

Electronic Engineer (with at least H.N.C. or equivalent and several years relevant experience), to design and build equipment for a research group—The Administrator, Clarendon Laboratory, The University, Oxford.

Entomologist (with a good honours degree in zoology or entomology, and preferably postgraduate research experience in forest or timber entomology) with the West African Timber Borer Research Unit, Kumasi, Ghana, to undertake studies of the biology, ecology and control of timber and lumber pests—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.197/510/01/C.1.

Head (with a degree in metallurgy, chemistry or a related science at the Ph.D. level or equivalent training with a number of years experience in the field of metal corrosion) of the Corrosion Section, Physical Metallurgy Division, Department of Mines and Technical Surveys, Canada, to initiate and supervise research and investigations on fretting corrosion, stress corrosion and corrosion fatigue of various metals and their alloys—Department 1517, National Employment Service of Canada, 61 Green Street, London, W.1.

Horticultural Advisory Officer (with a university degree in horticulture and postgraduate experience) in Malta, to develop cultivation of out-of-season flowers, fruit and vegetables primarily for export—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/19/07/T.

Lecturer (graduate physical chemist or metallurgist) in Extraction Metallurgy—Prof. F. D. Richardson, Department of Metallurgy, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7.

Lecturer in Physics at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

Lecturer (physicist, with experience and interest in high temperature gas physics) in the Department of Aerodynamics—The Recorder, The College of Aeronautics, Cranfield, Bletchley, Bucks.

National Research Council and other Post-Doctoral Fellowships, tenable in the Department of Physics—Prof. H. E. Duckworth, Chairman, Department of Physics, McMaster University, Hamilton, Ontario, Canada.

Scientific Officer (science graduate, preferably with honours in chemistry or biochemistry), for work on the preparation of blood products, including plasma protein fractions, with opportunity for research and developmental studies in an interesting and expanding field—The Director, Regional Blood Transfusion Centre, Royal Infirmary, Edinburgh 3.

Scientific/Senior Scientific Officers (with a first- or second-class honours degree in chemistry, or equivalent, and at least two years postgraduate research experience in organic chemistry, preferably with a bias towards the chemistry of natural products) at the Tropical Products Institute, D.S.I.R., London, W.C.1, to be members of small teams of research workers studying the isolation and structure of natural products possessing biological activity—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting Ref. F.849/9A.

Senior Lecturer in Pharmacology, and a Lecturer in Pharmaceutical Chemistry—The Registrar, Bradford College of Technology, Bradford 7.

Senior Cataloguer (chartered librarian, with experience in indexing scientific and technical information) in the College library—The Recorder, The College of Aeronautics, Cranfield, Bletchley, Bucks.

Senior Lecturer (qualified to direct and to teach to honours degree or Diploma of Technology levels, and preferably with industrial experience in a responsible position) in Textile Physics—The Registrar, Institute of Technology, Bradford 7.

Senior Physicist (with an honours degree in physics and experience in radiotherapy) with the Medical Research Council's Betatron Research Group, Christie Hospital and Holt Radium Institute, Manchester 20, to assist with the application of physics to the clinical use of the 4-MeV. Linear Accelerator and the 20-MeV. Betatron—Dr. Ralston Paterson, Christie Hospital and Holt Radium Institute, Manchester.

Technical Officer (man or woman, with an honours degree in mathematics, or in geography with a mathematical background) in the Survey Department, Government of Kenya, for survey computations and checking of survey plans—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.103/7/09.

Technician (Senior) (preferably with the Diploma of the Animal Technicians Association), to take charge of the Animal House—The Secretary, Guy's Hospital Medical School, London Bridge, S.E.1.

Technician (with a basic knowledge of, and an interest in, the maintenance and construction of electronic apparatus, and preferably experience in a physiological or pharmacological laboratory), to work in a new Department of Human Pharmacology—The Secretary, University College Hospital Medical School, University Street, London, W.C.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Transactions of the Royal Society of Edinburgh. Vol. 63, Part 2 (No. 25): The Mineralogy and Metamorphism of the Moine Schists of the Morar and Knoydart Districts of Inverness-shire. By Dr. R. St. J. Lambert. Pp. 553–588. 12*s.* 6*d.* Vol. 63, Part 3 (No. 26): *Staurastrum paradoxum* Meye Meyen and *S. gracile* Ralfs in the British Freshwater Plankton, and a revision of the *S. anatinum*-group of Radiate Desmids. By Dr. A. J. Brook. Pp. 589–628 (18 plates). 17*s.* 6*d.* Vol. 63, Part 3 (No. 27): A Structural History of the Girvan District, S.W. Ayrshire. By Dr. Alwyn Williams. Pp. 629–667. 21*s.* (Edinburgh: Royal Society of Edinburgh, 1959.) [191]

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Other Countries

NORDITA (Nordisk Organisation for Teoretisk Atomfysik. Årsberetning 1957/58 (Annual Report). Pp. 29. (København: Nordisk Institut for Teoretisk Atomfysik, 1959.) [161]

The Undiscovered Earth: Proceedings of the Conference, June 11–12, 1959. Pp. 56. (Birmingham, Alabama: Southern Research Institute, 200 Ninth Avenue South, 1959.) [161]

Kungl. Sjöfartstyrelsen, Stockholm. Ergebnisse der Beobachtungen des Magnetischen Observatoriums zu Lovö (Stockholm) in Jahre 195[illegible] Von Folke Eleman und KjellBorg. Pp. 30. (Stockholm: Sjöfarts styrelsen, 1959.) [161]

University College, Ibadan, Nigeria. Calendar, 1959–60. Pp. 9[illegible] (Ibadan: University College, 1959.) [191]

Canada: Department of Northern Affairs and National Resources: National Museum of Canada. Botanical Excursion to the Boreal Forest Region in Northern Quebec and Ontario. By W. K. W. Bladwin, E. Lapage, J. Terasmae, D. W. MacLean and I. J. Bassett. Pp. v+[illegible] Botanical Excursion to Jasper and Banff National Parks, Alberta: Alpine and Subalpine Flora. By A. E. Porsild. Pp. ii+38. The Native Flora of Churchill, Manitoba, with Notes on the History, Geology, and Climate of the Area. By H. J. Scoggan. Pp. vi+51. Bulletin No. 1[illegible] (Biological Series No. 58): Contributions to Botany, 1958. Pp. iii+[illegible] (3 plates). Natural History Papers, No. 3 (September 23, 1959): Anchiceratops from the Oldman Formation of Alberta. By W. Lan[illegible] ston, Jr. Pp. 11. (Ottawa: Queen's Printer, 1959.) [19]

National Science Foundation, Washington. Bibliography on the Economic and Social Implications of Scientific Research and Development. Pp. v+53. (NSF 59–41). 25 cents. Current Projects on Economic and other Impacts of Scientific Research and Development, 1959. Pp. v+59. (NSF 59–51). 25 cents. (Washington, D.C.: Government Printing Office, 1959.) [19]

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